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(54) PASSIVATED AND STABILIZED POROUS SUPPORTS AND METHODS FOR THE PREPARATION AND USE OF SAME

PASSIVIERTE UND STABILISIERTE TRÄGER UND METHODEN ZU DEREN HERSTELLUNG UND VERWENDUNG

SUPPORTS POREUX STABILISES ET RENDUS PASSIFS, ET LEUR PROCEDE DE PREPARATION ET D'UTILISATION

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Description

[0001] This invention relates generally to modified porous solid supports and processes for the preparation and use of same. In particular, passivated porous supports are disclosed which are characterized by a reversible high sorptive capacity substantially unaccompanied by non-specific adsorption of or interaction with biomolecules such as proteins, polysaccharides or oligo- or polynucleotides. Moreover, the passivated porous supports of the present invention exhibit other characteristics highly desirable in chromatographic applications, such as high porosity, physical rigidity, high charge density, and chemical stability under a variety of extreme conditions. The passivated porous supports of the present invention may also be used advantageously in a high flow, high efficiency mass transfer chromatographic technique which may be carried out in a fluidized-bed, packed-bed, or other mode of operation.

[0002] Polyfunctional macromolecules, such as proteins, can be purified by a variety of techniques. One of these techniques is known as ion-exchange chromatography. In ion-exchange chromatography, proteins are separated on the basis of their net charge. For instance, if a protein has a net positive charge at pH 7 it will bind to a negatively charged ion-exchange resin packed in a chromatography column. The protein can be released, for example, by decreasing the pH or adding cations that compete for binding to the column with the positively charged groups on the protein. Thus, proteins that have a low density of net positive charge, and thus a lower affinity for the negatively charged groups of the column, will tend to emerge first, followed by those having a higher charge density.

[0003] Generally, the ion-exchange resins which are used in these procedures are solids possessing ionizable chemical groups. Two types exist: cation-exchangers, which contain acidic functional groups such as sulfate, sulfonate, phosphate or carboxylate, and a second type, anion-exchangers, which contain functional groups such as tertiary and quaternary amines. These ionizable functional groups may be inherently present in the resin or they may be the result of the chemical modification of the organic or mineral solid support.

[0004] Organic ionic-exchangers which are made from polysaccharide derivatives, e.g., derivatives of agarose, dextran and cellulose, etc., have been used for both laboratory and industrial scale ion-exchange chromatography. However, these ion-exchangers have many disadvantages. First, polysaccharide-derived ion-exchangers are not very mechanically stable and are not resistant to strong acids. This instability limits the length of the column and, also, limits the flow rate through the column.

[0005] Second, such ion-exchangers have limited sorption capacity due to the limited number of ionic or ionizable groups that can be attached to the polysaccharide.

[0006] Third, these polysaccharidic derivatives are poor adsorbents for use in rapid fluidized-bed separations because of the low density of the material. In a fluidized bed it is desirable to pass the fluid without simultaneously washing out the particles. Therefore, it is generally desirable to have as great a density difference as possible between the solid support particles (e.g., silica) and the fluidizing medium.

[0007] The intrinsic high density of inorganic sorbents based on passivated mineral substrates facilitates packing and rapid decantation into chromatographic columns. Dense packing prevents formation of empty spaces and channeling when using packed beds. On the other hand, fluidization of dense particles in aqueous suspension is possible at high flow rates that, in turn, are very desirable when dealing with large scale applications. Operation of fluidized beds at high superficial flow velocities is generally not possible with low-density organic or polymeric sorbents, which can be elutriated from fluidized beds at relatively low liquid flow rates.

[0008] On the other hand, synthetic polymers are mechanically more stable than inorganic supports, and the former are more resistant to strong acidic conditions. However, they suffer disadvantages as well, such as limited capacity, limited solute diffusivity and thus, limited productivity. These synthetic polymers also suffer to some extent from the problem of non-specific adsorption of biomolecules, such as proteins. Untreated mineral supports such as silica are also inadequate in many chromatographic protein separation applications because of such non-specific adsorption.

[0009] Non-specific adsorption is caused by the interaction of a protein with the surface of the support -- be it organic or inorganic in nature. For example, silica is an acidic compound, and the negatively charged silanol groups present at the solid/liquid interface tend to create a separate ion-exchange interaction between the surface of silica and the protein. Non-specific adsorption is also caused by hydrogen bonding that takes place between, e.g. amino groups present in the amino acid residues of proteins and these same silanols present at the silica surface. Such non-specific interactions create separation problems during chromatography -- e.g., poor protein recovery and/or inadequate resolution. An important objective in the design of a chromatographic separation is generally to ensure a "single-mode" process of adsorption. However, the ion-exchange behavior associated with surface silanols can create a "mixed mode" adsorption system which makes the separation of biomolecules much more difficult. Although the sorption capacity generated by ionic silanol groups is low, the intensity of the interaction between the silanol groups and proteins can be high. These interactions therefore have the potential to cause denaturation of certain proteins.

[0010] Finally, both polysaccharides and most hydroxyl-containing synthetic sorbents are sensitive to the cleaning solutions used in industrial settings, which often include strong oxidizing agents such as hypochlorite or peracetic acid and which may be characterized by extremes of pH.

[0011] Thus, there is an important need for the development of improved passivation methods for the treatment of the surfaces of both polymeric and inorganic chromatographic supports in contact with protein-containing solutions, which method is capable of preventing or minimizing such non-specific interactions between proteins and the chromatographic support in order to improve the efficiency of chromatographic processes.

[0012] Several previous investigators have sought to passivate various microporous media including membranes and particulate chromatographic supports by applying thin surface coatings to inorganic or organic/polymeric substrates. For example, Steuck, in U.S. Patent No. 4,618,533, discloses a porous polymeric membrane substrate fashioned from a thermoplastic organic polymer upon which a permanent coating is grafted and/or deposited on the entire membrane surface. The polymerization and crosslinking of the polymerizable monomer upon and within the porous membrane substrate is performed in such a way that a thin coating is deposited upon the entire surface of the porous membrane, including the inner pore walls. Significantly, the porous configurations of the coated, composite membrane structures claimed by Steuck are essentially identical to those of the corresponding uncoated porous membrane substrates, implying that the polymer of Steuck is applied as a thin surface layer or coating that does not interfere with the porosity or flow properties of his composite membranes. Moreover, Steuck does not disclose the concept of a "passivating layer" or the use of monomers capable of functioning as "passivating" monomers within the meaning of the present invention as discussed in more detail below.

[0013] Varady et al., in U.S. Patent No. 5,030,352, disclose pellicular support materials useful as chromatography media which are obtained by applying various thin hydrophilic coatings to the surfaces of hydrophobic polymer substrates (e.g., polystyrene). Varady's surface coatings are applied by first exposing the surfaces of the hydrophobic substrate to a solution of a solute characterized by interspersed hydrophilic and hydrophobic domains; contact between surface and solute takes place under conditions that promote hydrophobic-hydrophobic interaction between solute and substrate, with the result that solute molecules are adsorbed onto the surface of the substrate as a thin coating that is ultimately crosslinked in place. Varady's coating materials may further comprise reactive groups capable of being derivatized to produce various materials useful in ion-exchange, affinity, and other types of chromatographic and adsorptive separations.

[0014] Significantly, however, the hydrophilic, functional coating of Varady's invention is limited to a thin adherent film on the surface of the hydrophobic support. The morphology of this coating layer is a direct and unavoidable consequence of the stated method of its deposition -- i.e., by the crosslinking of adjacent solute molecules adsorbed onto the surface of the hydrophobic substrate.

[0015] While Varady's coating method is at least partially effective in reducing the non-specific binding of proteins to the substrate, the sorption capacity of the chromatographic materials so produced is necessarily limited and inferior to those of the media produced by the process of the present invention. As discussed in considerably more detail below, the method of the present invention causes the formation of a crosslinked and functional gel that extends out into and substantially fills the pores of the support. As a consequence, the static and dynamic sorption capacities of the chromatographic media are not limited by the porous surface area of the substrate, as is the case with the pellicular materials of Varady's invention.

[0016] With regard to previous techniques for the passivation of inorganic or mineral supports by surface coating treatments, U.S. Patent No. 4,415,631 to Schutijser discloses a resin consisting of inorganic silanized particles onto which is bonded a crosslinked polymer comprised of copolymerized vinyl monomers and which contains amide groups. The invention specifies that the inorganic porous support, including silica, must be silanized prior to coating. The silanization treatment provides the inorganic porous support with reactive groups so that the copolymer can be covalently bonded to the silica surface.

[0017] Nakashima et al., in U.S. Patent No. 4,352,884, also discloses the use of silica as a porous substrate. The silica is coated with a polymer made up of acrylate or methacrylate monomer and a copolymerizable unsaturated carboxylic acid or a copolymerizable unsaturated amine. Nakashima et al. use an already preformed polymer to coat the support. Furthermore, Nakashima et al., in a separate and distinct step, utilize a crosslinking agent in a subsequent curing process.

[0018] The above-mentioned inventions are not completely successful, partly because of the unstable chemical linkage between the silica and the coating. The products of these inventions have the further disadvantages of not only failing to totally suppress the initial non-specific adsorption but also of introducing additional modes of non-specific adsorption.

[0019] Tayot et al., in U.S. Patent No. 4,673,734, disclose a porous mineral support that is impregnated with an aminated polysaccharide polymer that is said to cover the internal surface area of the support. However, since polysaccharides usually have very large molecular weights and their solutions are quite viscous, this process is not highly effective. Coverage of the entire internal surface of the silica substrate is problematic due to incomplete and uneven filling of the pores of the silica substrate by the large polysaccharide molecules.

[0020] The steric problems of Tayot's process result from the large size of the polysaccharides employed, the chains of which cannot penetrate completely within the pores of the support. This incomplete penetration results in the

creation of a "soft" layer of polysaccharide on the surface of the pore that subsequently causes problems during chromatographic separation. Polysaccharides such as dextran can also spontaneously hydrolyze at low pH, rendering them incompatible with certain cleaning operations that require the column or bed of chromatographic media to be washed with acid, alkaline, or oxidizing agents.

[0021] Despite these and other problems associated with the use of inorganic chromatographic suports, the use of mineral compounds such as silica as supports for chromatographic adsorbents is still attractive, because as explained above, chromatographic separations can be performed with such materials at very high flow rates -- for example, in very large-scale packed columns or in fluidized beds for industrial operations. What is needed are chromatographic supports characterized by high static and dynamic sorption capacity which exhibit improved chemical stability at alkaline and basic conditions and reduced tendencies to cause non-specific protein adsorption. It is an object of the present invention to provide such supports.

[0022] Accordingly, the present invention provides a passivated porous support comprising a porous solid matrix having interior and exterior surfaces and innate (i.e., inherently present) groups that render the matrix susceptible to undesirable non-specific interaction with biological molecules, and a three-dimensional pore-filling polymer network derived from a passivation mixture comprising effective amounts of an optional main monomer, a passivating monomer different from the main monomer, and a crosslinking agent, the mixture having been allowed to come into intimate contact with the surfaces of the matrix for a sufficient period of time such that on polymerization of the mixture the innate groups of the matrix become deactivated, resulting in the minimization or substantial elimination of the above-mentioned undesirable non-specific interactions.

[0023] The passivated porous supports of the present invention are further characterized by reversible high sorptive capacity for biological molecules including proteins. Furthermore, the passivated porous supports of the present invention enjoy exceptional chemical stability on exposure to strongly acidic or alkaline media and/or strong oxidizing solutions such as those that are frequently utilized during cleaning of industrial manufacturing equipment.

[0024] The primary objective of the present invention concerns the passivation of porous solid matrices that possess innate undesirable groups that render the matrix susceptible to non-specific interactions (e.g., adsorption) with biological molecules, in particular, proteinaceous substances.

[0025] A wide variety of non-passivated porous solid matrices are amenable to passivation by the general method of the present invention. These porous matrices include, but are not limited to, (i) mineral oxide supports, (ii) "stabilized" mineral oxide supports rendered chemically resistant to leaching by the application of thin protective coatings of hydrophobic polymers to their surfaces, and (iii) porous matrices comprised solely of organic/polymeric materials, in particular hydrophobic polymers. For example, mineral oxide supports, such as silica, alumina, and the like, may be transformed into passivated supports that exhibit desirable characteristics, such as high sorptive capacity, high density and good resolving (chromatographic) properties, unaccompanied by undesirable non-specific interactions that would otherwise be due largely to innate hydroxyl groups present on the surfaces of mineral oxides (e.g., silanols in the case of silica supports). It should be noted that transition metal oxides, such as zirconium, titanium, chromium and iron oxides are considered in the present invention to be within the scope of the term "mineral oxide" supports.

[0026] In the case of such mineral oxide supports, the non-specific interactions include either electrostatic interactions, hydrogen bonding, or both. Hence, the passivating monomer (alternatively described herein as the "neutralizing" monomer) is chosen to dampen, "neutralize," or "deactivate" such non-specific binding interactions; that is, one selects a passivating monomer that is capable of interacting with the innate groups of mineral oxide substrates either electrostatically or via hydrogen-bonding or both.

[0027] Moreover, in particular embodiments of the present invention, the passivating monomer can also act as the main monomer (i.e., said passivating or neutralizing monomer is chemically identical to the main monomer), but such situations are limited to those in which the neutralizing monomer is an acrylamide-based monomer that possesses at least one polar substituent, preferably an ionizable (e.g., tertiary amino, carboxylic acid, sulfonic acid, etc.) or ionic (e.g., ammonium, phosphate, etc.) substituent. In particular, acrylate-based monomers cannot serve both as the passivating (neutralizing) monomer and as the main monomer -- in part, because the acrylate-based monomers are less stable than the acrylamide-based monomers, particularly under strongly acidic or alkaline conditions.

[0028] Without wishing to be limited by theory, it is believed that the utilization of a passivating or neutralizing monomer, in combination with the optional main monomer and cross-linking agent, allows for the formation of a three-dimensional polymer network comprising a thin passivation region or layer that is substantially adjacent to the matrix surface, which polymer network extends into and throughout the porous volume of the substrate matrix and which passivation layer is made up primarily of units of the passivating or neutralizing monomer engaged in interactions with the innate groups of the substrate matrix. This thin passivation region or layer is additionally held in close proximity to the matrix surface by a lattice of optional main monomer units which extends from the passivation layer to the exposed exterior surfaces of the resulting "passivated" porous support. In addition, the cross linking agent acts to tether the respective polymeric (or copolymeric) chains to one another, thereby creating a stable three-dimensional polymer (i.e., "gel") network that is surprisingly effective in minimizing or eliminating undesirable non-specific binding interactions between

biological molecules and the non-passivated porous solid matrix.

[0029] Thus, it is also an object of the present invention to provide a passivated porous support comprising a porous solid matrix having interior and exterior surfaces and innate groups that render the matrix susceptible to undesirable, non-specific interaction with biological molecules, and a three-dimensional pore-filling polymer network derived from a passivation mixture comprising effective amounts of an acrylamide or methacrylamide monomer further substituted with at least one polar ionic or ionizable substituent, which monomer is capable of functioning both as a main monomer and as a passivating or neutralizing monomer, and a crosslinking agent, the mixture having been allowed to come into intimate contact with the surfaces of the matrix for a sufficient period of time such that on polymerization of the mixture, the innate groups of the matrix become deactivated, resulting in the substantial elimination of the above-mentioned undesirable non-specific interaction. Where porous matrices comprised of hydrophobic polymer substrates (as opposed to mineral oxide matrices) are concerned, it is a further object of the present invention to reduce the non-specific binding associated with exposure of such hydrophobic polymer surfaces to proteinaceous solutions. In particular, porous synthetic polymeric solid matrices comprised of such materials as polystyrene, polysulfone, polyethersulfone, polyolefins (e.g., polyethylene and polypropylene), polyacrylate, polyvinyl acetate (and partially hydrolyzed versions thereof), and the like, exhibit non-specific binding associated with hydrophobic-hydrophobic (among other types, e.g., hydrogen-bonding) interactions. Unlike the case of the mineral oxide matrix, in which the neutralizing monomer component of the passivating mixture is selected to deactivate polar groups like silanols, hydrophobic synthetic polymer matrices are passivated by the incorporation of passivating ("neutralizing") monomers that are capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction between them.

[0030] In a further object of the present invention, passivated porous supports exhibiting exceptional stability in alkaline media are provided. These passivated resins comprise porous solid matrices pre-coated with a thin film of a synthetic organic polymer, such as polystyrene or polystyrene substituted with nonionic, ionic, or ionizable functional groups. These pre-coated matrices exhibit the improved characteristics after being subjected to the passivation method disclosed herein.

[0031] More particularly, the methods of the present invention can be advantageously applied to the passivation of chromatographic support media comprised of porous mineral oxide particles (e.g., silica and alumina), the interior and exterior surfaces of which have previously been coated with a thin, protective layer of a coating polymer. This protective polymer coating is applied for the purpose of improving the chemical stability of the underlying mineral oxide material (e.g., against leaching or other chemical decomposition at alkaline, acidic, or strongly oxidizing conditions). For example, strongly alkaline aqueous media (e.g., 0.5 M sodium hydroxide solutions) are commonly used to clean chromatographic supports, and conventional silica supports can suffer significant weight loss (of order 50%) associated with leaching of the material over repeated cleaning cycles (e.g., 100 cycles).

[0032] The leaching of such unprotected mineral oxide supports gives rise to a number of problems, not the least of which is loss of mechanical integrity of the support and a consequent increase in the back pressure exhibited by columns packed with particles of the material. The problem of leaching can be addressed to some extent by using porous matrices characterized by lower surface areas (e.g., 5-10 m^2/g), but this is generally undesirable insofar as sorption capacity is often reduced by a corresponding amount.

[0033] The approach to substrate stabilization taken in one embodiment of the present invention involves coating the alkaline-sensitive porous mineral oxide substrate matrix with a soluble polymer that substantially encapsulates the mineral oxide matrix and thereby minimizes or prevents contact between the mineral oxide substrate and potentially destructive chemical cleaning solutions (e.g., caustic). The protective polymer coating is applied in the form of a thin surface layer upon the pore wall surfaces in order to avoid significantly decreasing the porous volume or blocking the mouths of pores. The protective polymer coating layer is readily applied, for example, by (i) first dissolving the protective polymer (e.g., polystyrene) in a suitable organic solvent to form a coating solution, (ii) subsequently impregnating the porous mineral oxide matrix with said solution, and then (iii) finally evaporating or otherwise removing the organic solvent.

[0034] While it has been discovered that this process of depositing protective polymer coatings upon the porous surfaces of mineral oxide (and particularly silica) matrices can significantly stabilize these materials by sharply reducing their rates of chemical leaching, the approach has the important disadvantage of rendering the porous surfaces of the coated and protected matrices hydrophobic and thus prone to cause excessive non-specific binding of proteins by adsorption. (This is precisely the same problem noted above in connection with entirely polymeric porous support matrices.) However, this problem can be successfully addressed by the methods of the present invention in the same way as the non-specific binding of strictly polymeric support matrices can be reduced -- i.e., by passivation in a process of oriented polymerization. More particularly, these composite chromatographic supports (i.e., supports comprised of min-

eral oxide substrates that have been stabilized by the application of thin protective polymer coatings) can be passivated against excessive non-specific binding by incorporating passivating ("neutralizing") monomers capable of associating with and consequently deactivating innate non-polar hydrophobic groups exposed on the matrix surface. The passivating monomers useful in this embodiment of the present invention adsorb upon (and consequently cover) the hydrophobic groups on the surface by virtue of their containing long-chain saturated hydrocarbons, olefinic hydrocarbon groups, aromatic groups, or like hydrophobic domains that interact with and become appreciably bound to their hydrophobic counterparts on the matrix surface as a consequence of the hydrophobic-hydrophobic interaction existing between them. Typically, the present invention utilizes base matrices having the following characteristics: an initial average particle size ranging from about 5 to about 1000 μm (microns); an initial porous volume ranging from about 0.2 to about 2 cm^3/gram; an initial surface area ranging from about 1 to about 800 m^2/gram; and an initial pore size ranging from about 5 nm to about 600 nm (about 50 to about 6000 angstroms). Preferably, the base matrix is characterized by: an initial average particle size ranging from about 10 to about 300 μm (microns), although passivated supports having narrow particle size ranges, such as about 15-20, about 15-25, about 30-45, about 50-60, about 80-100, and about 100-300 μm (microns), are most preferred Preferred ranges for other characteristics include an initial porous volume ranging from about 0.8 to about 1.2 cm^3/gram; an initial surface area ranging from about 10 to about 400 m^2/gram; and an initial pore size ranging from about 100 to about 300 nm (about 1000 to about 3000 angstroms). The density of the porous solid matrix obviously varies with its chemical nature, being higher for mineral oxide (e.g., silica) substrates and lower for polymeric ones (e.g., polystyrene).

[0035] The size exclusion limit varies somewhat from one type of passivated porous support to another, but generally falls in the range of about 500 to about 2,000,000 u (daltons), preferably, 50,000 to about 500,000. The sorptive capacity can also be manipulated, depending on the amount of main monomer incorporated in the polymer network, and ranges between about 1 milligram to about 300 milligrams of solute or biological molecule per unit volume (ml) of passivated support bed -- preferably at least about 50 mg/ml, and most preferably about 100 mg/ml.

[0036] Yet another object of the present invention relates to the passivation of non-passivated porous solid matrices while maximizing the openness (e.g., gel porosity and pore size) of the resulting passivated porous support. Such open gel morphologies have the advantage of permitting high sorption capacities to be achieved without affording excessive resistance to the transport of solutes such as proteins through the gel. Hence, in particular embodiments of the present invention, the polymerization of the passivation mixture is effected in the presence of an effective amount of a pore inducer.

[0037] A number of additives are suitable as pore inducers, including, but not limited to, polyethylene glycol, polyoxyethylene, polysaccharide, and the like. Also, the polymerization of the passivation mixture can be effected in the presence of an effective pore-inducing amount of a polar solvent. For example, the polymerization can be carried out in alcohol, a cyclic ether, a ketone, a tertiary amide, a dialkyl sulfoxide, or mixtures thereof. Preferably, such polar solvents include, but are not limited to, methanol, ethanol, propanol, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, acetone, dioxane, or mixtures thereof.

[0038] According to the present invention, polymerization is effected in the presence of an effective amount of a polymerization initiator, for example, thermal initiators such as ammonium persulfate/tertiary amine, nitriles or transition metals. Other examples include 2,2'-azobis(2-amidinopropane) hydrochloride, potassium persulfate/dimethylaminopropio-nitrile, 2,2'-azobis(isobutyronitrile), 4,4'-azobis(4-cyanovaleric acid), or benzoylperoxide. Photochemical initiators may also be used, such as isopropylthioxantone, 2-(2'-hydroxy-5'-methylphenyl) benzoltriazole, 2,2'-dihydroxy-4-methoxybenzophenone, riboflavin, and the like. Polymerization begins, as is known in the art, e.g., with agitation, exposure to heat, or exposure to a sufficient amount of radiant energy.

[0039] It is the object of the present invention to provide further passivated porous supports in which the optional main monomer of the polymer network comprises a vinyl monomer having at least one polar substituent. Such substituent may further be ionic, non-ionic, ionizable, or in the case of a vinyl monomer having more than one polar substituent, such substituents may be a combination of such substituents. It is preferred in affinity chromatography that the optional main monomer on polymerization, as part of the polymer network, have an affinity for a preselected biological molecule. However, the further modification of the polymer network to incorporate specific ligands capable of binding to biological molecules of interest is not precluded.

[0040] It should be apparent to one of ordinary skill in the art that the substituent(s) on the passivating or neutralizing monomer responsible for the "deactivation" (i.e., the reduction in the capacity of the innate groups of the non-passivated porous solid matrix to interact in a non-specific manner with biological molecules) should be tailored to the nature of the non-specific interaction to which the non-passivated porous solid matrix is susceptible. In essence, neutralizing monomers are provided which can interact with the innate groups of the matrix surfaces in the same manner as the non-specific interaction (e.g., electrostatically, via hydrogen bonding or both in the case of mineral oxide matrices -- or via hydrophobic-hydrophobic interaction in the case of synthetic polymeric matrices). Hence, substituents can be polar, cationic, anionic or hydrophobic depending on the particular application at hand. For example, suitable neutralizing monomers for porous mineral oxide matrices comprise a vinyl monomer having at least one polar ionic or ionizable

substituent. In one embodiment of the present invention, the substituent has the capacity to bear a positive charge. In particular, such neutralizing monomers are selected to provide near-surface passivating regions and polymer networks that are effective in deactivating polar groups on the surfaces of non-passivated matrices (e.g., in deactivating hydroxyl groups on the surfaces of porous mineral oxide matrices).

[0041] As a non-limiting example, neutralizing monomers useful in the passivation of porous mineral oxide matrices may be selected from diethylaminoethyl methacrylamide, diethylaminoethyl acrylamide, methacrylamido propyltrimethyl ammonium halide, triethylaminoethyl acrylamide, trimethylaminoethyl methacrylate, polyethyleneglycol dimethacrylate, dimethylaminoethyl methacrylate, polyethyleneglycol divinyl ether, or polyethyleneglycol diacrylate. Of these, the first four can function within the same composition both as a main monomer and a neutralizing monomer, as discussed above.

[0042] Likewise, suitable passivating monomers for use in the passivation of hydrophobic polymer surfaces -- whether said polymer is present as a protective surface coating on a mineral oxide matrix or as the bulk, structural material in the case of a porous polymeric chromatographic support matrix -- will typically comprise vinyl monomers having at least one substantially non-polar or hydrophobic substituent. In one embodiment of the present invention, this substituent comprises a hydrocarbon-rich functional group or moiety that imparts hydrophobicity to a portion of the passivating monomer.

[0043] In general, the hydrophobic character will result from the presence in the passivating monomer of a saturated (e.g., aliphatic) or unsaturated (e.g., aromatic) hydrocarbon substituent, and may further be described as straight-chain, branched, cyclic, or heterocyclic. Long-chain alkyl functional groups are particularly useful as substituents in this class of passivating monomers, which further contain one or more vinylic, acrylic, acrylamide, or allylic monomers. These passivating monomers are typically employed at concentrations in the reaction mixture of from about 0.1 to 1.0%.

[0044] Crosslinking agents useful in the present invention comprise vinyl monomers having at least one other polymerizable group, such as a double bond, a triple bond, an allylic group, an epoxide, an azetidine, or a strained carbocyclic ring. Preferred crosslinking agents having two double bonds include, but are not limited to, N,N'-methylenebis-(acrylamide), N,N'-methylenebis(methacrylamide), diallyl tartradiamide, allyl methacrylate, diallyl amine, diallyl ether, diallyl carbonate, divinyl ether, 1,4-butanedioldivinylether, polyethyleneglycol divinyl ether, and 1,3-diallyloxy-2-propanol.

[0045] It is a further object of the present invention to provide a method of passivating a porous solid matrix having interior and exterior surfaces and innate groups that render the matrix susceptible to undesirable non-specific interaction with biological molecules, comprising: (a) contacting the surfaces of the matrix with a passivation mixture comprising effective amounts of an optional main monomer, a neutralizing monomer different from the main monomer, and a crosslinking agent; and (b) effecting the polymerization of the mixture to form a three-dimensional polymer network within the pores of the matrix, such that the innate groups of the matrix become deactivated, resulting in the substantial elimination of undesirable non-specific interaction.

[0046] In the present method the amount of neutralizing monomer is chosen to be sufficient to counteract the innate groups present on the surface of the non-passivated matrix. Furthermore, the surfaces of the matrix are contacted (e.g., by dropwise addition) with a solution of the passivation mixture. Generally, the passivation mixture is prepared as an aqueous solution and, as mentioned above, may in addition contain effective amounts of a pore inducer. In a preferred embodiment of the present invention as it is applied to porous mineral oxide matrices, the volume (in ml) of the passivation mixture solution is adjusted to correspond approximately to the weight (in grams) of the non-passivated porous solid matrix.

[0047] Yet another object of the present invention is related to a method of separating a desired biological molecule from a sample containing same comprising: (a) loading a column packed with the passivated porous support having an affinity for a preselected biological molecule with a sample containing the preselected biological molecule; and (b) passing an eluent solution through the loaded column to effect the separation of the preselected biological molecule. The sample may be introduced to the column in any number of ways, including as a solution. Chromatographic separations employing these passivated supports in fluidized-bed modes of operation are also within the scope of the invention.

[0048] The methods of the present invention are effective to isolate or separate a broad range of biological molecules, including peptides, polypeptides, and proteins (such as insulin and human or bovine serum albumin), growth factors, immunoglobulins (including IgG, IgM, and therapeutic antibodies), carbohydrates (such as heparin) and polynucleotides (such as DNA, RNA, or oligonucleotide fragments).

[0049] Eluent solutions suitable for use in the present invention are well known to those of ordinary skill in the art. For example, a change in ionic strength, pH or solvent composition may be effective in "stepwise" elution processes. Alternately, eluent solutions may comprise a salt gradient, a pH gradient or any particular solvent or solvent mixture that is specifically useful in displacing the preselected biological molecule. Such methods are generally known to those engaged in the practice of protein chromatography. Still another object of the present invention relates to a chromatographic method for the separation of biological molecules comprising passing a sample containing a mixture of biolog-

ical molecules through a column packed with the passivated porous support disclosed herein.

[0050] Moreover, a method of preparing a passivated porous solid support is disclosed comprising: (a) contacting a porous solid matrix, having interior and exterior surfaces and innate groups that render the matrix susceptible to undesirable non-specific interaction with biological molecules, with a passivation mixture comprising effective amounts of an optional main monomer, a passivating or neutralizing monomer different from the main monomer, and a crosslinking agent; and (b) effecting the polymerization of the mixture to form a polymer network within the pores of said porous solid matrix, such that the innate groups of the matrix become deactivated, to provide a passivated porous solid support that is substantially free of undesirable non-specific interactions.

[0051] These and other objects of the present invention will become apparent to those skilled in the art from a reading of the instant disclosure.

Fig. 1A is a graph which schematically represents the chromatographic separation of a protein mixture consisting of (1) cytochrome, (2) bovine hemoglobin, (3) ovalbumin, and (4) beta-lactoglobin on a cationic passivated porous support. The conditions of the experiment were as follows:

- Column Size	1.0 cm ID x 7.8 cm
- Initial Buffer	50 mM Tris-HCl, pH 8.6
- Elution gradient	0-1 M NaCl
- Flow Rate	125 ml/h

Fig. 1B is a graph which schematically represents the chromatographic separation of a protein mixture consisting of (1) ovalbumin, (2) beta-lactoglobulin, (3) cytochrome c, and (4) lysozyme on an anionic passivated porous support. The conditions of the experiment were as follows:

- Column Size	1.0 cm ID x 7.5 cm
- Initial Buffer	50 mM Acetate, pH 6.5
- Elution Gradient	0-2 M NaCl
- Flow Rate	150 ml/h

Fig. 2 represents a comparison between the chromatographic separations of a protein mixture consisting of (1) ovalbumin, (2) beta-lactoglobulin, (3) cytochrome c, and (4) lysozyme using an anionic passivated porous support and an anionic nonpassivated matrix. The conditions of the experiment were as follows:

- First Buffer	acetate 50 ml, pH 6.5
- Second Buffer	acetate 50 ml, pH 6.5 2 M NaCl, pH 4.5
- Flow Rate	140 ml/h

Fig. 3A shows a graph of useful relative sorption capacity versus flow rate for various porous supports including the porous supports of the present invention passivated with a cationically charged polymer network (i.e., a passivated porous support useful as an anion-exchange resin).

Fig. 3B shows a graph of productivity versus flow rate for the various porous supports shown in Fig. 3A.

Fig. 4 shows a graph of the absolute sorptive capacity (in mg/ml) as a function of flow rate of a variety of solid supports, including a passivated porous support of the present invention.

Fig. 5 is a schematic illustration of the putative architecture of the three-dimensional polymer network formed within and extending from the internal surfaces of an individual pore in a porous solid matrix upon polymerization of the passivation mixture of the present invention.

[0052] The process of the present invention requires first the dissolution of monomers in water or in an aqueous/organic solution.

[0053] A primary component of the passivation mixture of the present invention is the main monomer, which may be omitted and is, therefore, optional. The appropriate amount of main monomer (or other solute) for use in the present invention is expressed as a percentage equal to the number of grams of main monomer per 100 ml of monomer solution (percent weight/volume). For purposes of the present discussion, the volume of the monomer solution is effectively the volume of the solution of a passivation mixture containing optional main monomer, neutralizing monomer, and crosslinking agent. Appropriate concentrations of the main monomer range from about 5% to about 50% (i.e., 5-50 grams of main monomer per 100 ml of monomer solution). Preferred concentrations of the main monomer are from about 7% to about 20%.

[0054] For purposes of this application, the main monomer is defined as including any monomer known to those skilled in the art which can be utilized for the preparation of an adsorbent useful in a chromatographic separation (e.g., affinity, ion-exchange, and the like). Such monomers include, but are not limited to, non-ionic monomers, ionic monomers, hydrophilic monomers, hydrophobic monomers, and reactive monomers. Reactive monomers are monomers having special functional groups that enable them to react chemically with other molecules that are subsequently immobilized irreversibly on the polymer network. This procedure is the basis of affinity chromatography, the chemically attached molecule being referred to as the "ligand." The main monomers of the present invention can be aliphatic, aromatic or heterocyclic; however, they must possess a polymerizable double bond; for example, the main monomers can be acrylic, allylic, vinylic or the like.

[0055] More specifically, anionic polymers are used to create anionic sorbents (i.e., cation-exchange supports). The functional groups (i.e., the substituents on the vinyl monomer) are preferably: carboxylic groups (e.g., acrylic acid, N-acryloylaminohexanoic acid, N-carboxymethylacrylamide), sulfonate groups (e.g., acrylamidomethyl-propane sulfonic acid), or phosphate groups (e.g., N-phosphoethyl-acrylamide).

[0056] Cationic polymers used to create cationic sorbents may contain the following functional groups: substituted amino groups (e.g., diethylaminoethyl methacrylamide, diethylaminoethyl acrylamide, methacrylamidopropyltrimethylammonium halide, triethylaminoethyl acrylamide, trimethylaminoethyl methacrylate, polyethyleneglycol dimethacrylate, dimethylaminoethyl methacrylate, polyethyleneglycol divinyl ether, or polyethyleneglycol methacrylate), or heterocyclic amines (e.g., 2-vinylpyridine, vinylimidazole, 4-vinyl-pyridine). Nonionic polymers may be comprised of: acrylamide, hydroxy-containing acrylamide derivatives (e.g., N-tris-hydroxymethylmethyl-acrylamide, methylolacrylamide, dimethylacrylamide, 2-hydroxyethylacrylamide, N-acryloyl-morpholine), methacrylamide, hydroxy-containing methacrylamide derivatives, heterocyclic neutral monomers (e.g., vinylpyrrolidone, N-acryloylmorpholine), or hydroxy-containing acrylates and methacrylates (e.g., hydroxyethylacrylate or hydroxyethyl methacrylate, hydroxyphenyl methacrylate, 4-vinylphenol, and 2-hydroxypropylacrylate).

[0057] Hydrophobic monomers useful in creating sorbents for hydrophobic chromatography include octyl-acrylamide or -methacrylamide, phenyl-acrylamide, butyl-acrylamide, benzyl-acrylamide, and triphenylmethyl-acrylamide.

[0058] Activated monomers useful in creating preactivated sorbents (i.e., those that can be further derivatized directly with a "ligand") for affinity chromatography include glycidyl-acrylate or - methacrylate, acrolein, acrylamidobutyraldehyde dimethylacetal, acrylic-anhydride, acryloyl chloride, N-acryloxysuccinimide, and allyl-chloroformate.

[0059] The passivation mixture further comprises an appropriate amount of a passivating or neutralizing monomer capable of neutralizing the non-specific adsorption properties of innate sites on the surface of the porous solid support. In the case of silica, the acidic character of innate silanol groups proves problematic during separations, and it is thus desirable to neutralize these silanol groups. The amount of neutralizing monomer to be used is preferably an amount sufficient to counteract approximately up to an equivalent number of Si-OH groups present at the exterior and interior surfaces of said support. The amount of neutralizing monomer, again expressed as a percentage (weight/volume), should be about 0.5% to about 6% (w/v), preferably about 1.5% to about 3% (i.e., about 1.5-3 grams of neutralizing monomer per 100 ml of monomer solution).

[0060] Suitable neutralizing monomers for use in the present invention may be monomers bearing a positive charge at a neutral pH; examples include monomers containing a cationic amine group, such as substituted amines or pyridine and the like. The cationic neutralizing monomers must have at least one double bond, such as vinyl, acrylic, or allylic monomers.

[0061] To counteract the acidic character of silica and its tendency to form hydrogen bonds, cationic monomers or monomers which are able to engage in hydrogen bonding (dipolar interactions) are also useful as neutralizing monomers in a particular embodiment of the present invention.

[0062] Preferred neutralizing cationic monomers of the present invention include, but are not limited to, diethylaminoethyl acrylamide, diethylaminoethyl methacrylamide, diethylaminoethyl methacrylate, methacrylamide propyltrimethyl ammonium halide, triethylaminoethyl acrylamide, triethylaminoethyl methacrylate and copolymers thereof.

[0063] Polyoxyethylene-containing monomers can also be used. This latter group can interact with polar groups (via hydrogen bonding). Preferred neutralizing monomers able to induce hydrogen bonding are polyoxyethylene monomers

like poly(ethylene glycol)$_n$-dimethylacrylate, where "n" is between about 50 and about 1000.

[0064] Preferred neutralizing hydrophobic monomers include, but are not limited to, N-alkylacrylamide in which the alkyl groups are branched, N-alkylacrylamide methylene chains having up to about 20 carbon atoms in the alkyl moiety, and N-arylacrylamide derivatives, like N-benzylacrylamide, N,N-(1,1-dimethyl-2-phenyl)ethyl-acrylamide, N-triphenyl methylacrylamide, or N,N-dibenzyl acrylamide. Specific representative passivating monomers useful in treating polymeric or polymer-coated matrices include, but are not limited to, N-tert-octylacrylamide (TOA), N-(1-methylundecyl)-acrylamide (MUA), N-(1,1,3,5-tetramethyl)-octylacrylamide (TMOA), Triton-X-100-methacrylate (TWMA), and polyethyleneglycol-dimethacrylate (PEG-DMA). Hydrophobic adsorption sites present on the internal surfaces of some organic (i.e., polymeric) porous matrices like polystyrene -- or on protective polymer coatings deposited on porous mineral oxide matrices -- are neutralized using hydrophobic passivating monomers incorporating these aromatic and aliphatic hydrophobic moieties or substituents.

[0065] To the mixture comprising the neutralizing and optional main monomers, a bifunctional crosslinking agent is added. The crosslinking agent allows the three-dimensional insoluble polymeric network to form within the pore volume of the porous matrix. In the absence of the crosslinker called for in this invention, the polymer formed would be linear and thus soluble. The amount of crosslinking agent should be about 0.1% to about 10% (w/v). Alternatively, the amount of crosslinking agent can be calculated based on the total weight of main monomer and neutralizing monomer in use. Preferably, the amount of crosslinking agent is from about 3 to about 10 percent by weight of the total weight of main and neutralizing monomers.

[0066] The crosslinking agents used in the present invention are acrylic, vinylic or allylic monomers that possess at least two polymerizable functional groups. Preferred crosslinking agents have at least two double bonds and include, but are not limited to, N,N'-methylene-bis-acrylamide, N,N'-methylene-bis-methacrylamide, diallyl tartradiamide, allyl methacrylate, diallyl amine, diallyl ether, diallyl carbonate, divinyl carbonate, divinyl ether, 1,4-butanedioldivinylether, and 1,3-diallyloxy-2-propanol.

[0067] Thereafter, said mixture is admixed with a porous solid matrix, thereby filling the pores of the matrix. As regards inorganic support materials, suitable porous mineral oxide matrices used in the present invention include but are not limited to silica, alumina, transition metal oxides (including but not limited to titanium oxide, zirconium oxide, chromium oxide, and iron oxide) and any other similar ceramic material including silicon nitride and aluminum nitride. The preferred mineral moieties of the present invention include silica, zirconium oxide, and titanium oxide. The most preferred mineral moiety is porous silica of a particle size of about 5 µm to about 1000 µm, having a porous volume of about 0.2 to about 2 cm^3/g, a pore size of about 5 to about 600 nm (about 50 to about 6000 Å), and a surface area of about 1 to 800 m^2/g. At this time, most all of the aqueous solution will have been absorbed by the mineral support, leaving a substantially dry, solid porous matrix.

[0068] After filling the pores of the porous mineral oxide matrix, (e.g., silica) with the aqueous solution of monomers (preferably, the volume of the solution expressed in mls is approximately equal to the weight in grams of the silica matrix), the mixture is placed in a non-aqueous dispersing medium. Suitable non-aqueous media include non-polar organic solvents known to those skilled in the art. Such non-aqueous media for suspending the treated matrix may include, but are not limited to, mineral and vegetable oils, aromatic solvents, aliphatic low molecular weight solvents, or chlorinated solvents. The most preferred non-aqueous media include toluene, methylene chloride, and hexane.

[0069] Thereafter, a polymerization starter is added to the mixture, now in a non-aqueous medium, in order to initiate polymerization of the monomers within the silica pores. The concentration of initiator (expressed as percent weight per volume of initial monomer solution) is from about 0.1% to about 2%, preferably about 0.8% to about 1.2%.

[0070] It should be apparent to those of ordinary skill that certain initiators are best dissolved in aqueous media while others can be dissolved in organic media. Hence, depending on the solubility characteristics of a particular initiator or combination of initiators, the polymerization initiator can be added to the initial solution of passivation mixture prior to addition of that mixture to the porous solid matrix. In particular, an initiator combination of ammonium persulfate and tetramethylethylenediamine (TMEDA) can be introduced separately. One component (the water-soluble persulfate salt) is combined with the aqueous mixture of optional main monomer, neutralizing monomer, and crosslinking agent, while the other component (TMEDA) is combined with the non-aqueous dispersing medium.

[0071] It should be noted that the persulfate/TMEDA combination is particularly useful because TMEDA displays appreciable solubility in water. Hence, in the dispersion comprised of the treated support, water and non-aqueous solvent, the TMEDA is able to penetrate the pores of the treated support and thereby initiate polymerization, particularly upon heating.

[0072] Typical polymerization initiators known to those skilled in the art can be used in the present invention. For instance, these initiators may be capable of generating free radicals. Suitable polymerization starters include both thermal and photoInitiators. Suitable thermal initiators include, but are not limited to, ammonium persulfate/tetramethylethylene diamine (TMEDA), 2,2'-azobis-(2-amidino propane) hydrochloride, potassium persulfate/dimethylaminopropionitrile, 2,2'-azobis(isobutyro-nitrile), 4,4'-azobis-(4-cyanovaleric acid), and benzoyl-peroxide. Preferred thermal initiators are ammonium persulfate/tetramethyethylenediamine and 2,2'-azobis(isobutyroni-

trile). Photoinitiators include, but are not limited to, isopropylthioxantone, 2-(2'-hydroxy-5'-methyl-phenyl)benzotriazole, 2,2'-dihydroxy-4-methoxybenzophenone, and riboflavin. It is further contemplated that riboflavin be used in the presence of TMEDA. When using the combination of persulfate and tertiary amine, the persulfate is preferably added prior to the addition of the non-aqueous medium, since persulfate is much more soluble in water than in non-aqueous dispersing media.

[0073] In another embodiment, the polymerization step can take place in the presence of a pore inducer. The pore inducers of the present invention allow polymerization to take place without substantially reducing the porosity of the solid support. Suitable pore inducers, also referred to as porogens, used in the present invention include, but are not limited to, polyethylene glycols, polyoxyethylenes, polysaccharides such as dextran, and polar solvents. Polar solvents include those commonly used in chemical synthesis or polymer chemistry and known to those skilled in the art. Suitable polar solvents include alcohols, ketones, tetrahydrofuran, dimethylformamide, and dimethysulfoxide. Preferred polar solvents are ethanol, methanol, dioxane, and dimethysulfoxide.

[0074] Porous polymeric matrices amenable to passivation by the methods of the present invention include, but are not limited to, polystyrene, polysulfone, polyethersulfone, various cellulose esters (e.g., cellulose acetate, cellulose nitrate), polyolefins (e.g., polyethylene and polypropylene), polyvinylacetate (and partially hydrolyzed versions thereof), polyacrylates, polyvinylidene fluoride, polyacrylonitrile, polyamides, polyimides, and various blends, mixtures, and copolymers thereof. Procedures for the manufacture of porous particles and other structures (e.g., microporous membranes) from such polymers are generally known in the art.

[0075] Where the polymer surface to be passivated is in the form of a thin, protective coating residing upon the pore walls of mineral oxide substrate that is thus stabilized against leaching, the polymer will generally consist of a linear, high-molecular-weight polymer capable of being dissolved in a suitable organic solvent. For example, a coating solution of linear polystyrene (e.g., with an average molecular weight 400 Ku (kilodaltons)) is conveniently prepared by dissolving the polymer in a chlorinated hydrocarbon such as methylene chloride. Typical concentrations of polymer in the coating solution range from about 2% (w/v) to about 20% (w/v). The ideal concentration is determined by achieving a balance between effectiveness in preventing or minimizing leaching of the mineral oxide substrate (which argues for higher polymer concentrations) and the constriction of pores and partial loss of porous volume (and sorption capacity) that can occur at higher polymer concentrations. Where protective coatings of polystyrene are deposited on porous silica, a polystyrene concentration of about 10% (w/v) is preferred. The coating is applied by first impregnating the porous support with the solution of protective coating and then removing the solvent vehicle by evaporation.

[0076] Certain modifications to the passivation procedures employed with porous mineral oxide matrices are indicated where the exposed surface of the porous matrix to be passivated is a polymeric one -- i.e., in those cases where (i) the porous support particle is fashioned entirely of a polymer or (ii) a mineral oxide matrix is protected by a stabilizing polymer coating. In these situations, polymerization of the passivating mixture by the process described above, entailing the dispersion of the porous particles (impregnated with aqueous monomer solution) in a non-aqueous (i.e., "oil-phase") dispersing medium, has certain disadvantages. The problems stem from the fact that the surfaces of polystyrene-coated silica and other polymer-coated mineral oxide matrices are predominantly hydrophobic and compatible with oil-phase dispersing agents that would otherwise be used in the polymerization step. Oil-phase dispersing media are prone to penetrating the pores of matrices that present exposed polymeric surfaces, and the presence of oil inside the pores causes various manufacturing problems (e.g., partial solubilization of the coating polymer, difficulty in effecting removal of the oil from the pores, etc.).

[0077] Accordingly, a modified polymerization procedure is advantageously employed where polymeric surfaces are to be passivated, which procedure entails a so-called "dry polymerization" procedure as opposed to that described above involving an oil-phase dispersing medium. In particular, the porous matrix impregnated with aqueous passivating mixture (i.e., monomer solution) undergoes the polymerization reaction while in the form of an apparently "dry" and free-flowing powder, typically agitated (e.g., by stirring or fluidization) in a closed, inert (e.g., nitrogen) atmosphere. The dry polymerization reaction is typically conducted at a temperature from about 60 to 90 °C, at a pressure of 1 to 2 bars, and for a period ranging from about 2 hours to overnight.

[0078] Suitably "dry" but monomer-solution-impregnated powders can be prepared by adding the aqueous passivating mixture in a careful, metered fashion (e.g., dropwise) to the porous matrix, so that little or no excess liquid-phase passivating mixture is present. The incorporation of organic cosolvents (e.g., ethanol, dimethylsulfoxide, and the like) in the monomer mixture assists the process of wetting the polymeric or polymer-coated mineral oxide matrix by the predominantly aqueous passivation mixture. For example, the crosslinking agent is conveniently added to the final monomer mixture in the form of an aqueous 10% ethanol solution.

[0079] Because no oil-phase is present as a dispersing medium in this embodiment of the invention, the initiators (i.e., polymerization catalysts) employed in this dry polymerization process are necessarily water-soluble and are generally thermally activated. A representative thermally-activated polymerization initiator is azo-bis-amidinopropane.

[0080] In yet another aspect of the invention, polymeric and polymer-coated mineral oxide matrices may be treated with hydrophilic polymers such as polyoxyethylene (POE) and polyvinylpyrrolidone (PVP) prior to effecting the polym-

erization and crosslinking of the monomer solution within the pores of the support. Treatment in this manner can be effective in reducing non-specific-binding interactions with proteins even in the absence of the oriented polymerization of hydrophobically binding passivating monomers present in the monomer soluton. Without wishing to be limited as to theory, it is believed that such high-molecular-weight passivating polymers are initially adsorbed upon the surfaces of the polymeric or polymer-coated mineral oxide matrix. Upon polymerization of the monomer solution, these polymers become substantially immobilized by the formation of an interpenetrating polymer network. That is, the POE or PVP polymer becomes entrapped in a "sandwich" type of structure between the pore-wall surface and the three-dimensional polymer lattice that occupies most of the porous volume.

[0081] In all cases, i.e., whether the porous matrix is comprised of a mineral oxide, a polymer-coated and thus stabilized mineral oxide, or a polymer, the polymerization process of the present invention creates a three-dimensional lattice or crosslinked polymer network that extends away from the pore-wall surfaces of the porous solid matrix. Again, not wishing to be limited by theory, it is believed that this polymer network is comprised of a thin passivating region or layer that interacts with the surface of the non-specific adsorption sites of the solid support (e.g., silanols in the case of silica) covalently linked with a three-dimensional structural polymer lattice that substantially fills the porous volume. The three-dimensional shape of the polymer lattice is believed to be substantially identical to the shape of the pore which it fills (see Figure 5), with the passivating layer oriented adjacent to and continuous (i.e., covalently linked) to the three-dimensional polymer lattice that extends away from the matrix surface. This configuration prevents "neutralizing" or "deactivating" pieces of the polymer network from eluting from the support during regular use -- for example, when the passivated porous support is exposed to vigorous washing or cleaning conditions, such as high acidic pH, high alkaline pH, high ionic strength, and strong oxidizing conditions. This crosslinked polymer network creates a novel chromatographic sorbent which can then be used, for example, in a process for separating and purifying various biomolecules, including macromolecules.

[0082] Indeed, it has been surprisingly discovered that the passivated porous supports of the present invention manifest chromatographic characteristics that are unparalleled under several criteria, particularly in terms of dynamic sorptive capacity as a function of flow rate. In particular, whereas the great majority of porous materials suffer a marked decrease in useful sorptive capacity as flow rates increase (e.g., at flow rates of about 50 cm/h or greater), the passivated porous supports of the present invention show little decrease in useful sorptive capacity from a static condition up to flow rates approaching 200 cm/h. Compare, for example, the behavior of prior art "gel"-type materials with the supports of the present invention, as illustrated in the graphs of Fig. 3A, 3B, and 4 (described further in Example 16).

[0083] Moreover, the absolute capacities of the passivated porous supports of the present invention are considerably greater than even those attained with other types of solid supports (e.g., Spherodex™) that exhibit a similar insensitivity to high flow rates. Thus, as shown in Fig. 4, a plot of the absolute capacity vs. flow rate of various solid supports unambiguously shows that the passivated solid supports of the present invention combine a very high absolute sorption capacity (expressed as mg/ml) with a relative insensitivity to solution flow rates.

[0084] It is believed, without wishing to be limited by theory, that a highly open, flexible lattice structure comprised primarily of polymeric chains of repeating main monomer units is formed within the pores of the porous solid matrix. Very significantly, it is believed that the areas of the porous support available for desirable reversible interaction with biological molecules are not confined to the regions immediately adjacent to the surface of the pore as is the case when thin, substantially two-dimensional coatings are applied to porous surfaces in the manner of Steuck (U.S. Patent No. 4,618,533) and Varady et al. (U.S. Patent No. 5,030,352) as discussed in Section 2.2 above. Rather, it is believed that the polymeric network of the present invention extends outwardly into the pore volume itself in the manner of a three-dimensional lattice, as opposed to a two-dimensional coating limited strictly to the pore wall surface area. A schematic diagram of such a structure, as it is thought to exist, is illustrated in Fig. 5, where a biological molecule of interest (depicted as a spherical object) is also shown interacting with the lattice. Furthermore, the presence of porogens (pore-inducers) in the passivation mixture is believed to promote creation of this open three-dimensional polymer network.

[0085] It is further thought that such an extended polymer network contributes not only to the unusually high absolute sorptive capacity of the passivated solid supports of the invention as measured under static (i.e., no flow) conditions, but also allows the present invention to maintain such high sorptive capacities largely independent of solution flow rates. It is thought that perhaps the open, flexible nature of the three-dimensional polymer network allows biological molecules to rapidly penetrate the polymer lattice and thereby efficiently interact with sorptive groups in the polymer network of the passivated porous support even at high solution flow rates. The rapid and efficient mass transfer of biomolecules into and through this network avoids the decrease in useful or dynamic sorption capacity and resolution that are typical of conventional chromatographic media. With these conventional media, diffusion in the pores of the support and/or materials coated thereupon or within them leads to poor mass transfer rates and limits the efficiency of the chromatographic process.

[0086] Thus, a method of performing chromatographic separations characterized by high sustained sorptive capacity independent of flow rate and rapid, efficient mass transfer is achieved with the passivated porous supports of the present invention, which supports include an open, flexible three-dimensional network or lattice of crosslinked polymer

chains extending within and throughout the pores of the support matrix.

[0087] The separation and purification process usually involves at least two steps. The first step is to charge a packed or fluidized bed column containing the passivated porous solid support with a solution containing a mixture of biomolecules, at least one of which it is desired to separate and recover in at least partially purified form. The second step is to pass an eluent solution through said column to effect the release of said biomolecules from the column, thereby causing their separation.

[0088] "Stepwise" elution can be effected, for example, with a change in solvent content, salt content or pH of the eluent solution. Alternatively, gradient elution techniques well known in the art can be employed. For instance, proteins reversibly bound to cation exchange media can generally be eluted by increasing the pH to alkaline values (subject to limits associated with the chemical stability of the protein), and immunoglobulins bound to protein A or like adsorbents may be eluted by decreasing the pH to acidic values.

[0089] The invention is further defined by reference to the following examples that describe in detail the preparation of the passivated porous solid support and the methods of using the same. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the purpose and scope of this invention.

[0090] To better understand the procedures described in the following examples, several terms are defined for the benefit of the reader, below.

The *passivation level* is an estimation of the absence of non-specific adsorption of a strong cationic molecule like lysozyme, which characteristically forms very strong complexes with silanols on the silica surface.

Porosity factor is the ratio between elution volume (V_e) of a protein (e.g., BSA in our case) and the total volume (V_t) of the packing bed determined under physiochemical conditions (e.g., high ionic strength) in which no interactionx-ists between the protein and the porous support.

Sorption capacity is the amount of adsorbed protein in "mg" per unit volume (ml) of passivated porous support bed determined under particular conditions:

- for cationic sorbents: 50 m M Tris-HCl, pH 8.6.
- for anionic sorbents: 50 mM Acetate, pH 6.5.

Ion exchange capacity is the number of ionizable groups in µeq per unit volume (ml) of passivated porous support bed determined by titration.

EXAMPLE 1: Preparation of a porous cation-exchange resin.

[0091] 20 grams ("g") of acrylamidomethyl propane sulfonic acid (AMPS) sodium salt and 1 g of N,N'-methylene-bis-acrylamide (MBA) are dissolved in 50 ml of distilled water. 3 g of diethylaminoethyl methacrylamide, are added and then the pH of the total solution is adjusted to between 6 to 8 to make a final solution volume of the passivation mixture of 100 ml. To this solution of monomers, 500 mg of ammonium persulfate are added at room temperature.

[0092] While shaking, the solution of monomers is added dropwise to 100 g of porous silica (40 to 100 µm diameters, 100 to 150 nm (1000 to 1500 Å) pore diameter, 20 to 35 m^2/g surface area and 1 cm^3/g porous volume).

[0093] After 30 minutes of shaking, 250 ml of paraffin oil is added, the agitated suspension is heated at 60 to 70 °C and then 1 ml of N,N,N',N'-tetramethylethylene diamine is added.

[0094] After a few minutes, the exothermic polymerization reaction occurs. The resin is then separated by a chlorinated solvent and dried at room temperature. Lastly, the resin is washed extensively with dilute hydrochloric acid, dilute sodium hydroxide and 1 M sodium chloride.

[0095] This cation-exchange resin shows the following characteristics:

- A titration curve with an acidic pK due to the presence of sulfonic acid groups;
- No presence of anionic groups which are oriented on the acidic silanols of the silica surface.
- A number of acidic groups of 395 µeq/ml.
- A sorption capacity for insulin in 70% ethanol of about 80 mg/ml.
- An exclusion limit of about 30 Ku (Kd).

EXAMPLE 2: Preparation of an anion-exchange resin.

[0096] 20 g of methacrylamidopropyl trimethyl ammonium chloride (MAPTAC) and 1 g of N,N'-methylene-bis-acrylamide (MBA) are dissolved in 80 ml of distilled water and the pH of the solution is adjusted to 7.5. Separately, 1 g of ammonium persulfate is dissolved in 20 ml of distilled water. The two solutions were then mixed together at room tem-

perature.

[0097] While shaking, the monomer solution is added dropwise to 100 g of dry porous silica (40-100 µm bead diameter, 100-150 nm (1000-1500 Å) porous volume, 20-35 m^2/g surface area and 1 cm^3/g porous volume).

[0098] After shaking for about 30 minutes, 250 ml of paraffin oil is added and the mixture heated at 60-70 °C. 2 ml of N,N,N',N'-tetramethylenediamine is added to polymerize the monomer solution inside the silica pores.

[0099] The same recovery and washing steps are performed as those described in Example 1.

[0100] The obtained resin shows the following characteristics:

- Ion-exchange capacity: 114 µeq of quaternary ammonium groups per ml of resin.
- No visible presence of acidic (silanol) groups on titration curve.
- No non-specific adsorption of cytochrome c at pH below its isoelectric point.
- Sorption capacity for bovine serum albumin (BSA): 91 mg/ml resin.
- Porosity factor for BSA (V_e/V_t): 0.52.

EXAMPLE 3: Preparation of a second anion-exchange resin using different amounts of crosslinker.

[0101] Three 80 ml solutions each containing two monomers (MAPTAC and MBA) are prepared according to Example 2, using varying amounts of MBA: 0.5 g, 1 g and 2 g.

[0102] All other operations are identical to Example 2. The anion-exchange resins differ by the following properties:

Amount of MBA	0.5 g	1 g	2 g
Ionic charges per ml of resin	36 µeq	114µeq	218µeq
Sorption capacity per ml (BSA)	35 mg	91 mg	72 mg

EXAMPLE 4: Preparation of an anion-exchange resin using MBMA as a crosslinker.

[0103] 1 g of N,N'-methylene-bis-methacrylamide (MBMA) is dissolved in 50 ml of dimethylsulfoxide (DMSO). To this mixture 40 ml of an aqueous solution containing 20 g of MAPTAC is added.

[0104] While stirring, 1 g of ammonium persulfate previously dissolved in 10 ml of distilled water is added. The obtained monomer solution is then used to fill the silica pores (1 cm^3/g porous volume; 120-150 nm (1200-1500 Å) pore diameter) and the resin is prepared according to the previous examples except toluene is used as the non-aqueous solvent instead of paraffin oil.

[0105] The obtained anion-exchange resin shows the following characteristics:

- Ion-exchange capacity: 201 µeq of quaternary amino groups per ml of resin.
- Sorption capacity for BSA: 112 mg/ml.
- No non-specific adsorption of cationic proteins like cytochrome c are present.
- Porosity factor for BSA (V_e/V_t): 0.53

EXAMPLE 5: Preparation of anion-exchange resins with a different amount of MBMA.

[0106] Three different resins are prepared according to Example 4 with differing amounts of MBMA as a crosslinking agent.

[0107] When 100 ml of a DMSO-water solution is used, the amount of MBMA is varied as follows: 0.5 g, 1 g and 2 g. Paraffin oil is used as the non-aqueous (organic) solvent at 60 °C.

[0108] The obtained resins show the following characteristics:

Amount MAPTAC	20g	20g	20g
Amount MBMA	0.5g	1g	2g
Ionic charges per ml of resin	168µeq	212µeg	231µeq

(continued)

Sorption capacity per ml	114	106	76
Porosity factors for BSA (V_e/v_t)	0.52	0.52	0.51

[0109] It is demonstrated that the amount of crosslinking agent does not modify the porosity of the three dimensional polymer at least within the explored zone. The amount of ionic groups which depends on the amount of the main monomer remains also quite constant.

[0110] All of the above resins are stable to oxidizing agents, such as hypochlorites and peraceticacid.

EXAMPLE 6: Preparation of strong cationic exchangers using silicas of different porosity.

[0111] 7g of AMPS, 3 g of MAPTAC and 1 g of MBA are dissolved in 100 ml of distilled H_2O. 1 g of ammonium persulfate is then added and the solution is divided into two parts of 50 ml each. Separately, each solution is added to 50 g of dry silica having the following properties listed in the table below:

	Particle Size	Surface Area	Porous Volume	Pore Diameter
Assay a	40-100 µm	25 m^2/g	1 cm^3/g	125 nm (1250 Å)
Assay b	40-100 µm	10 m^2/g	1 cm^3/g	300 nm (3000 Å)

[0112] All other operations are performed according to Example 1.

[0113] The following are the final properties of the cationic exchangers:

	Assay a	Assay b
Ionic charges per ml	92 µeq	89 µeq
Sorption capacity (cytochrome c)	86 mg	81 mg
Non-specific absorptions	negative	negative

[0114] This example demonstrates that the available porosity is independent of the silica quality. The choice of silica is more linked to its sensitivity to an alkaline media. For example, the alkaline sensitivity of silica having a surface area of 5 m^2/g is 50% lower than when using a sample having a surface area of 25 m^2/g.

EXAMPLE 7: Preparation of cation-exchangers using different amounts of anionic monomer.

[0115] The aqueous solutions of monomers (100 ml) are composed of:

MAPTAC	3 g (monomer to neutralize the silanol groups of silica)
AMPS	7 g and 10 g (varying amounts of anionic monomer)
MBA	1 g (crosslinker)

[0116] All other operations (mixing with silica, polymerization and recovery) are identical to those described on Example 1.

[0117] The final properties of the final cation-exchangers obtained are as follows:

- Quantity of AMPS	7 g	10 g
- Ion-exchange groups per ml	92µeq	147µeq
- Sorption capacity per ml (cytochrome c)	86 mg	120 mg

[0118] This example confirms that when the amount of functionalized monomer in the initial solution is increased, the number of ion-exchange groups is proportionately higher. The sorption capacity for cytochrome c increases as well.

EXAMPLE 8: Preparation of a strong cation-exchange resin with MBMA as crosslinker.

[0119] 0.5 g of MBMA are dissolved in 50 ml of DMSO while stirring. To this solution 30 ml of aqueous solution containing 10 g of AMPS is added as well as 6 ml of a 50% aqueous solution of MAPTAC.

[0120] The final volume is adjusted to 100 ml prior the addition of 1 g of ammonium persulfate at room temperature.

[0121] This solution of monomers is added dropwise to 100 g of dry porous silica to fill completely the available porous volume (1 cm^3/g for a pore size of 125 nm (1250 Å). The remaining operations are identical to the method described in Example 1. The final cation-exchange resin shows the following characteristics:

- Ion-exchange groups per ml of resin	123 µeq
- Sorption capacity for cytochrome c	128 mg
- Porosity factor for lysozyme	0.82
- Resistance to oxidizing agents (NaOCl)	Excellent even at a concentrated form (1/10 dilution of commercial) concentrated product.

EXAMPLE 9: Preparation of a weak cation-exchange resin.

[0122] In 60 ml of distilled water, 6 ml of a 50% aqueous solution of MAPTAC, 1 g of MBA and 10 ml of acrylic acid are dissolved.

[0123] The volume of the solution is then adjusted to 100 ml, the pH adjusted to about 4.5, and 1 g of ammonium persulfate is added at room temperature.

[0124] As described for other examples the solution of monomers is added to 100 g of porous silica and then polymerized in a non-aqueous water-immiscible solvent (e.g., paraffin oil, toluene, or methylene chloride).

[0125] The final characteristics of the resin are as follows:

- Ion-exchange groups (carboxylates) per ml	337 µeq
- Sorption capacity for cytochrome c	118 mg
- Non-specific adsorption (chromatographic test)	Excellent

EXAMPLE 10: Preparation of non-ionic hydroxyl-containing resins for immobilization of biologicals.

[0126] The monomers comprising the initial solution are the following:

-Tris-hydroxymethyl-methylmethacrylamide (THMMA)	Non ionic monomer
-MAPTAC or DEAE methacrylamide	cationic monomer to neutralize the silanol groups.

(continued)

-MBA	crosslinking agent

[0127] The composition of the solutions are:

	Assay a	Assay b	Assay c
THMMA	10 g	10 g	20 g
MAPTAC	1.5 g	-	2.5 g
DEAE-methacrylamide	-	2 g	-
MBA	2 g	3 g	2 g

[0128] All other operations (mixture with dry silica, polymerization and recovery) are identical to those described in previous examples.

[0129] The final characteristics of the resins are:

- Good passivation of the silica surface. No significant amount of cationic proteins adsorbed in normal conditions of gel filtration.
- V_e/v_t for bovine albumin is respectively 0.71, 0.74 and 0.61.

[0130] After chemical modification the resin is utilized to immobilize either a dye (Cibacron Blue F3GA) or heparin.

[0131] Each affinity sorbent is very effective to purify human albumin and antithrombin III, respectively, in a single pass.

EXAMPLE 11: Preparation of a cationic resin in the presence of polyethylene glycol as a pore inducer.

[0132] Two monomer solutions are prepared as described in Example 8. A solution of 10 g of polyethylene glycol 6000 is added to one.

[0133] Final volumes are adjusted to 100 ml, pH adjusted to about 7 and then 1 g of ammonium persulfate is added to both solutions.

[0134] The monomer mixture is added to porous silica 120 nm (1200 Å) pore diamater, 40-100 µm particle diameter, 25 m^2/g surface area), polymerization and recovery are effected as described in previous examples. The obtained resins show the following characteristics:

	+PEG-6000 (10%)	PEG-6000
MAPTAC	20 g	20 g
MBMA	1 g	1 g
CATIONIC GROUPS (µeq/ml)	200	193
SORPTION CAPACITY BSA	112	127
V_e/V_t β-lactoglobulin	0.578	0.511
V_e/V_t BSA	0.548	0.513
V_e/V_t Immunoglobulins G	0.495	0.481

[0135] This example demonstrates that, in spite of the same amount of initial material (similar number of ionic groups), the porosity is influenced by the presence of PEG-6000.

[0136] The exclusion limit is actually larger when PEG is added.

EXAMPLE 12: Further separations of protein mixtures by ionic resins.

[0137] Two resins are used to show their ability to separate protein mixtures rapidly and efficiently:

- a cationic resin (quaternary ammonium resin from Example 5).
- an anionic sulfonated resin (see Example 8).

[0138] The cationic resin (201 µeq quaternary amino groups/ml) is packed in a column of 1 cm in diameter and 8 cm in length and then equilibrated with a 0.05 M Tris-HCl buffer, pH 8.5. A sample containing 1 mg of cytochrome c, hemoglobin, betalactoglobulin and ovalbumin is injected and separated under a salt gradient.

[0139] The results of the separation of the four components is given below (Fig. 1A). Separation is achieved under a flow rate of 120 ml/hour.

[0140] The anionic resin (138 µeq SO_3 groups/ml) is packed in a column of 1 cm in diameter and 7 cm in length and then equilibrated with a 0.05 M acetate buffer, pH 4.5. A sample containing ovalbumin, betalactoglobulin, cytochrome c, and lysozyme is injected and separated under a salt gradient.

[0141] The result of the separation of four components is given below (Fig. 1B). Separation is achieved under a flow rate of 140 ml/hour.

EXAMPLE 13: Demonstration of the need to neutralize the silanol group when preparing a cation-exchange resin.

[0142] Two aqueous solutions of monomer (100 ml each) are prepared according to Example 1 differing essentially by the presence of the cationic monomer MAPTAC.

[0143] Final composition of monomer solutions is as follows:

	Assay a	Assay b
AMPS	10 g	10 g
MBMA	0.5 g	0.5 g
MAPTAC	3 g	0

[0144] All the operations (mixing with silica, polymerization and recovery) are identical to those described in the above-mentioned examples.

[0145] The final properties of the obtained cation-exchangers are as follows:

	Assay a	Assay b
Ion-exchanger groups per ml	123 µeq	118 µeq
Sorption capacity per ml (cyt.c)	128 mg	77 mg
Separation efficiency	excellent (see fig. below)	no separation

[0146] This result demonstrates the necessity to neutralize acidic silanols that disturb the separation mechanism.

EXAMPLE 14: Influence of the amount of cationic monomer on the passivation of silica surface.

[0147] To demonstrate that the amount of cationic monomer necessary to neutralize silanol groups (passivation is proportional to the surface area) a series of trials are effected with porous silicas with different surface area.

[0148] Silicas chosen are the following:

	Silica X 015	Silica X 075
Surface area per g	25 m^2	100 m^2
Porous volume per g	1 cm^3	1 cm^3
Bead size	40-100 µm (microns)	40-100 µm (microns)

[0149] Trials are performed using different amounts of MAPTAC (cationic monomer) copolymerized with a non-ionic acrylic monomer (THMMA).

[0150] After polymerization, the degree of passivation is estimated by the measurement of non-specific adsorption of lysozyme.

TABLE I

COMPOSITION OF POLYMERS AND RELATED ANALYTICAL RESULTS								
Type of silica	X 075	X 075	X 075	X 075	X 075	X 015	X 015	X 015
Surface area/g	100 m^2	100 m^2	100 m^2	100 m^2	100 m^2	25 m^2	25 m^2	23 m^2
Amount MAPTAC	0 %	1.5 %	3 %	6 %	12 %	0 %	1.5 %	3 %
Crosslinking ratio	0 %	10 %	10 %	10 %	10 %	0 %	10 %	10 %
Non-specific ads. (Lysozyme)	55 mg	13 mg	13 mg	0 mg	0 mg	15 mg	0 mg	0 mg
Passivation ration level	-	±	+	+	++	-	+	++

++ Indicates that the number of non-specific absorptions is close to zero, indicating an excellent passivation level.

+ At a non-specific adsorption of less than 10 mg, passivation is also quite good.

± Indicates that the passivation level is less than 15 mg, which in most instances is not acceptable for use in chromatographic separation.

- Indicates that the passivation level is greater than 15 mg and thus the material is not performing the separation function correctly and thus cannot be used for chromatographic separation.

[0151] It is thus demonstrated that the level of non-specific adsorption for lysozyme (a strong cationic protein) is high when the MAPTAC is absent. The non-specific adsorption for silica with large surface ares (X 075, 100m^2/g) is higher (55 mg/ml of resin) than the non-specific adsorption for silica X 015 (25 m^2/g; 15 mg/ml of resin). A certain proportionality exists between the surface area and the original level of non-specific absorptions. The amount of MAPTAC to decrease the level of non-specific adsorption down to zero is also proportional to the surface area available: 1.5% of MAPTAC is necessary with silica X 015 (25 m^2/g) whereas at least 6% is necessary to passivate silica X 075 (100 m^2/g).

EXAMPLE 15: Preparation of an Anion Exchange Resin Based on Polystyrene.

[0152] 10 g of methacrylamidopropyltrimethylammonium chloride, 2 g of N-(1,1-dimethyl-2-phenyl)ethylacrylamide and 2 g of N,N,-methylene-bis-methacrylamide are dissolved in 30 ml of dimethyl sulfoxide. The volume of the solution is then increased to 50 ml by adding 20 ml of water. Under stirring, 0.3 g of 2,2,-azobis-(2-amidinopropane)- hydrochloride is added at room temperature.

[0153] While shaking, the monomer solution is added dropwise to 50 g of porous polystyrene (50-150 µm beads diameter, 30-40 nm (300-400 Å) pore diameter). The excess of monomer solution is thus eliminated by filtration under vacuum. The impregnated polystyrene beads are introduced into a closed container and heated at 80-90 °C for five hours to polymerize the monomer solution within the pores of the polystyrene matrix.

[0154] Finally the obtained material is washed extensively with ethanol to eliminate the excess monomers and, subsequently, with water.

[0155] The resulting resin showed the following characteristics:

- Very hydrophilic material (in opposition to the totally hydrophobic nature and unwettability, of the polystyrene

- Ion exchange capacity: 100 µeq/ml of resin
- Sorption capacity for BSA: 70 mg/ml.

EXAMPLE 16: Performance Characteristics of the Passivated Porous Support of the Present Invention at High Flow Rates

[0156] The performance characteristics of the passivated porous support are compared with those of other support materials under high solution flow rates (e.g., approaching 100 cm/h). In particular, the relative sorption capacity and productivity characteristics of DEAE-Spherodex™, DEAE-Trisacryl Plus™, DEAE-Trisacryl™, DEAE-Agarose-based sorbent, and passivated porous supports of the present invention are illustrated in Figs. 3A and 3B. The absolute sorption capacities at flow rates approaching 200 cm/h are compared for these supports in Fig. 4. The data of Fig. 4 are generated for a 50 mM Tris buffer (pH 8.6) solution of BSA (5 mg/ml).

[0157] It can be seen from Fig. 3A, that the useful sorption capacity decreases by half or more at flow rates between about 50 cm/h to about 100 cm/h for Trisacryl, Trisacryl Plus and the Agarose-based sorbent. By contrast, the degree to which the useful sorption capacity of the passivated porous supports of the present invention (e.g., the passivated support of Example 2 or 4) is retained as flow rate increases compares favorably with DEAE-Spherodex™ even at flow rates approaching 100 cm/h (i.e., the useful sorption capacity remains substantially unchanged as a function of flow rate).

[0158] Moreover, the productivity, a measure of the amount of material processed in the separation procedure per unit time, of the respective supports are compared in Fig. 3B. Again, the performance of the passivated porous supports of the present invention compares favorably with the DEAE-Spherodex™ sorbent. The passivated porous supports of the present invention are clearly superior to DEAE-Spherodex™, however, when their sorption capacities are compared on an absolute basis, as shown in Fig. 4.

EXAMPLE 17: Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Precoated) Silica Passivated Porous Support

[0159] Polystyrene pellets (10 g, average molecular weight about 400,000 u (daltons) are dissolved in 100 ml of methylene chloride and then added dropwise to 100 g of porous silica (400-100 µm diameter, 200-300 nm (2000-3000 Å) pore diameter, 10 m^2/g surface area and about 1 cm^3/g porous volume). After about 30 minutes shaking the mixture is dried under an air stream at room temperature until total evaporation of the chlorinated solvent (i.e., until a constant weight is observed). The obtained dry powder is then heated at 190 °C overnight to permit the polystyrene to form a homogeneous thin layer on the surfaces (internal and external) of the silica.

[0160] Next, 20 g of methacrylamidopropyl trimethyl ammonium chloride (MAPTAC) and 1 g of N,N'-methylenebismethacrylamide are dissolved in 80 ml of distilled water and the pH of the solution is adjusted to 7.5. Separately, 1 g of ammonium persulfate is dissolved in 20 ml of distilled water. The two solutions are then mixed together at room temperature and added dropwise to 100 g of polystyrene-coated silica, obtained as described above. After shaking for about 30 minutes, paraffin oil (250 ml) is added to the mixture, along with 2 ml of N,N,N',N'-tetramethylethylenediamine to polymerize the monomer solution inside the silica pores. The resulting suspension is then heated at 60-70 °C to induce polymerization.

[0161] The passivated resin is then recovered by filtration. The oil is eliminated with an extensive washing with water containing 0.1-0.5% of a non-ionic detergent and then stored in a saline buffer at neutral pH. The product resin shows very similar ion-exchange characteristics as those described in Example 2. Additionally, its sensitivity in strong alkaline media is much improved as measured by its weight loss after one night of contact with 0.5 M sodium hydroxide. The passivated resin of this example lost only about half as much weight as an anionic resin prepared from silica having an unprotected surface area.

[0162] Alternatively, the polystyrene can be coated on the surfaces of the matrix by polymerizing the vinyl monomer in situ, thus assuring that the internal surfaces of even the smallest pores of the matrix are coated with protective polymer. The conditions for the polymerization of the vinyl monomer are well known to those of ordinary skill (e.g., see, Kirk-Othmer Concise Encyclopedia of Chemical Technology, Wiley-Interscience Publication, New York, pp. 1115-1117). After such an in situ polymerization, it is preferred that the coated support be heated overnight at 190 °C, as described above, to provide a homogeneous thin-film layer over the matrix.

[0163] In addition, the polystyrene may also contain substituents, particularly at the 4-position of the phenyl ring, which can be non-ionic or ionizable. For example, carboxylic acids, carboxylic acid esters or amides, sulfates, phosphates, N,N-dialkylcarboxamides, lower alkylamines, N,N-dialkylamines, quaternary ammonium groups, and the like can be present on the polymer. Indeed, a 4-iodo substituent on all or a portion of the phenyl groups of polystyrene would allow a large host of other functional group to be introduced by known methods (e.g., formation of aryllithium, Grignard, or copper reagents followed by quenching with carbon dioxide or alkylation).

[0164] Moreover, passivation of the porous solid matrix having a thin-film coating of a synthetic organic polymer can also be achieved by other variations in the procedure disclosed in the present invention, such as the method of Example 15.

EXAMPLE 18: Determination of Ion-Exchange and Protein Sorption Capacity of Preparation of Anion-Exchange Resins Based on Passivated Porous Silica Support of Different Surface Areas

[0165] This example provides evidence that polymerization of the passivation mixture within porous silica matrices forms a three-dimensional polymer network or "lattice", as opposed to a thin, substantially two-dimensional surface coating. Three anion-exchange sorbents were prepared using the methods of the present invention, the differences between the sorbents relating primarily to the pore sizes and hence internal surface areas of the silica matrices. These silica substrate characteristics are summarized in the following table:

	X-005	X-015	X075
Particle size (µm (microns))	40-100	40-100	40-100
Porous volume (cm^3/g)	1	1	1
Pore size (nm (Angstroms))	300(3000)	125(1250)	30(300)
Surface area (m^2/g)	10	25	100

[0166] Surface area is seen to increase as the pore size decreases, while porous volume remains essentially constant.

[0167] The characteristics of the passivated ("Q-CPI") anion-exchange support prepared from these silica base materials are summarized in the following table:

Silica Matrix	X-005	X-015	X-075
Particle size (µm (microns))	40-100	40-100	40-100
Ionic groups (microeq/ml)	111	133	183
BSA capacity (mg/ml)	130	125	82
Sorption efficiency	1.17	0.94	0.45

[0168] The ion-exchange capacity (i.e., number of ionic groups) and BSA sorption capacity are seen to be relatively constant; in fact, these values decrease somewhat as the surface area of the silica support is increased). In particular, ion-exchange and BSA sorption capacities do not increase as the surface area of the silica increases (i.e., from left to right in the table). This supports the interpretation that the polymeric lattice formed upon polymerization of the passivating solution forms a three-dimensional, substantially pore-filling network, as opposed to a thin pore-wall surface coating.

EXAMPLE 19: Preparation of an Anion-Exchange Resin Based on a Surface-Protected (i.e., Polystyrene-Precoated) Passivated Porous Silica Support

[0169] Polystyrene pellets (10 g, average molecular weight approximately 400 Ku (kD) were dissolved in 10 ml of methylene chloride and then added dropwise to 100 g of porous silica. The silica was characterized by a particle diameter of 40 to 100 µm (microns), a pore diameter of 200 to 300 nm (2000 to 3000 Angstroms), a surface area of 10 m^2/g surface area, and a porous volume of about 1 cm^3/g. After about 30 minutes of shaking, the mixture was dried under an air stream at room temperature until total evaporation of the chlorinated solvent had occurred, as evidenced by the attainment of a constant particle weight. The dry powder was then heated overnight at 180 °C to permit the polystyrene to form a thin, homogeneous surface layer or coating on both the internal and external exposed surface regions of the silica. This polystyrene-coated silica so obtained exhibited only a fraction of the sensitivity to alkaline media that was

exhibited by unprotected silica matrices. In particular, deposition of the protective polystyrene coat in this manner was observed to reduce the extent of silica leaching by a factor of at least 2 to 3.

[0170] Next, 0.5 g of N-1-methylundecyl-acrylamide (MUA) were dissolved in 100 ml of pure ethanol, and the solution was added dropwise to 100 g of the polystyrene-coated silica obtained as described above. After shaking for about 30 minutes, the material was placed in a nitrogen stream under conditions that resulted in complete evaporation of the ethanol (again, as observed by attainment of constant solids weight).

[0171] Next, 1 g of N,N'-methylene-bis-methacrylamide was dissolved in 20 ml of dimethylsulfoxide. To this solution, 20 g of methacrylamidopropyltrimethylammonium chloride (MAPTAC) were added, and the total volume of the solution was adjusted to 80 ml by the addition of distilled water. Separately, 0.5 g of azo-bis-amidino-propane (as initiator) was dissolved in 10 ml of distilled water and then added to the solution of monomers. The volume of the latter was then adjusted to 100 ml with water; 90 ml of this solution were then added dropwise to the polystyrene-precoated silica.

[0172] This material (i.e., monomer-solution-impregnated polystyrene-precoated silica) was then placed under nitrogen and in a closed vessel at 80 °C for over two hours. The product so obtained was then washed extensively with water and water-compatible solvents to remove any unpolymerized material and other reaction byproducts.

[0173] The cationic (i.e., anion-exchange) resin so prepared exhibited a fixed-charge density (i.e., ion-exchange capacity) of 150 microequivalents/ml of quaternary amino groups. Its capacity for reversibly absorbing BSA was 125 mg/ml. Non-specific binding (expected to be extensive and excessive for unpassivated, polystyrene-coated silica) was minimal for the material produced by the method of the present invention.

EXAMPLE 20: Preparation of a Cationic Resin Based on a Porous Polystyrene Matrix

[0174] Porous polystyrene beads, characterized by a particle diameter of 50 to 70 µm (microns), a pore diameter of 100 nm (1000 Angstroms), and a porous volume of 1.6 cm^3/g, were obtained as a commercially available product from Polymer Laboratories, Inc. (Amherst, MA). Five grams of these porous crosslinked polystyrene beads were washed extensively with ethanol and then dried under vacuum.

[0175] Separately, 61 mg of methylene-bis-methacrylamide were dissolved in 3.76 ml of dimethyl sulfoxide. To this was added 2.44 ml of an aqueous solution containing 1.3 g of methacrylamidopropyltrimethylammonium chloride (MAPTAC) and 25 mg of azo-bis-amidino-propane. To this solution, which was stirred gently under a nitrogen atmosphere at 4 °C, was added 1.5 ml of pure ethanol. This solution was then added dropwise to the dry polystyrene beads until it was totally absorbed within the porous volume of the beads. After 30 minutes of shaking, the mixture was stirred in a closed vessel under a nitrogen pressure at 85 °C for at least 2 hours. After this period, the product beads were removed and washed extensively with acidic, alkaline, and aqueous alcohol solutions to remove reaction byproducts and uncopolymerized materials.

[0176] The anion-exchange resin product obtained in this manner was very hydrophilic and contained cationic groups at a density of 124 microequivalents/ml of settled resin volume. Protein sorption capacity as measured by uptake of bovine serum albumin (BSA) was between 30 and 50 mg/ml of settled resin, depending on operating conditions.

EXAMPLE 21: Preparation of a Passivated Cationic Resin Based on a Porous Polystyrene Matrix

[0177] Example 21 differs from the preceding Example 20 in its incorporation of the passivating monomer MUA into the mixture polymerized within the pores of the polystyrene support. As before, porous polystyrene beads, characterized by a particle diameter of 50 to 70 µm (microns), a pore diameter of 100 nm (1000 Angstroms), and a porous volume of 1.6 cm^3/g, are obtained as a commercially available product from Polymer Laboratories, Inc. (Amherst, MA). Five grams of these porous crosslinked polystyrene beads are washed extensively with ethanol and dried under vacuum.

[0178] Separately, 61 mg of methylene-bis-methacrylamide are dissolved in 3.76 ml of dimethyl sulfoxide. To this are added 2.44 ml of an aqueous solution containing 1.3 g of methacrylamidopropyltrimethylammonium chloride (MAPTAC) and 25 mg of azo-bis-amidino-propane. To this solution, which is stirred gently under a nitrogen atmosphere at 4 °C are added 1.5 ml of pure ethanol containing 50 mg of N-1-methyl-undecyl-acrylamide (MUA) as a passivating ("neutralizing") monomer. This solution is then added dropwise to the dry polystyrene beads until it is totally absorbed within the porous volume of the beads. After 30 minutes of shaking, the mixture is stirred in a closed vessel under a nitrogen pressure at 85 °C for 2 hours or more. After this period, the product beads are removed and washed extensively with acidic, alkaline, and aqueous alcohol solutions to remove reaction byproducts and uncopolymerized materials.

[0179] The anion-exchange resin product obtained in this manner contains cationic groups at a density of about 115 microequivalents/ml of settled resin volume. Protein sorption capacity as measured by uptake of bovine serum albumin (BSA) is about 80 mg/ml of settled resin. The resin is stable over a wide range of pH values (from 1 to 14) and can be used advantageously in the chromatographic separation of various protein mixtures.

EXAMPLE 22: Preparation of a Passivated anionic Resin Based on Porous Polystyrene Matrix

[0180] Example 22 differs from the preceding Example 21 in two respects: (i) its replacement (on a 1-for-1 basis by weight) of an anionic monomer (acrylamido-methyl-propane sulfonic acid sodium salt) for the cationic monomer (MAPTAC) used in the passivating mixture polymerized within the pores of the porous polystyrene support, and (ii) its use of N-(1,1,3,5-tetramethyloctyl)-acrylamide as opposed to N-1-methyl-undecyl-acrylamide (MUA) as the passivating or neutralizing monomer.

[0181] As before, porous polystyrene beads, with a particle diameter of 50 to 70 µm (microns), a pore diameter of 100 nm (1000 Angstroms), and a porous volume of 1.6 cm^3/g, are obtained from Polymer Laboratories, Inc. Five grams of these porous crosslinked polystyrene beads are washed extensively with ethanol and dried under vacuum.

[0182] Separately, 61 mg of methylene-bis-methacrylamide are dissolved in 3.76 ml of dimethyl sulfoxide. To this are added 2.44 ml of an aqueous solution containing 1.3 g of acrylamido-methyl-propane sulfonic acid sodium salt and 25 mg of azo-bis-amidino-propane. To this solution, which is stirred gently under a nitrogen atmosphere at 4 °C, are added 1.5 ml of pure ethanol containing 50 mg of N-(1,1,3,5-tetramethyloctyl)-acrylamide as a passivating ("neutralizing") monomer. This solution is then added dropwise to the dry polystyrene beads until it is totally absorbed within the porous volume of the beads. After 30 minutes of shaking, the mixture is stirred in a closed vessel under a nitrogen pressure at 85 °C for 2 hours or more. After this period, the product beads are removed and washed extensively with acidic, alkaline, and aqueous alcohol solutions to remove reaction byproducts and uncopolymerized materials.

[0183] The cation-exchange resin product obtained in this manner is very hydrophilic and contains anionic (sulfonate) groups at a density of about 100 microequivalents/ml of settled resin volume. Protein sorption capacity as measured by uptake of lysozyme is about 95 mg/ml of settled resin. The anionic resin is stable over a wide range of pH values (from 1 to 14) and can be used advantageously in the chromatographic separation of various protein mixtures.

EXAMPLE 23: Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Precoated) and POE-Passivated Porous Silica Support

[0184] Polystyrene pellets (10 g, average molecular weight approximately 400 Ku (kD) were dissolved in 10 ml of methylene chloride and then added dropwise to 100 g of porous silica. The silica was characterized by a particle diameter of 40 to 100 µm (microns), a pore diameter of 200 to 300 nm (2000 to 3000 Angstroms), a surface area of 10 m^2/g surface area, and a porous volume of about 1 cm^3/g. After about 30 minutes of shaking, the mixture was dried under an air stream at room temperature until total evaporation of the chlorinated solvent had occured, as evidenced by attainment of a constant particle weight. The dry powder was then heated overnight at 190-200 °C.

[0185] This polystyrene-coated silica was then suspended in 200 ml of an aqueous solution of 5% polyoxyethylene (POE) with an average molecular weight of about 600 Ku (kD). The mixture was stirred gently for about 5 hours at 85°C and then the excess solution was removed by filtration. The silica beads were then washed extensively with water to remove the excess POE; the beads were finally rinsed twice with pure ethanol and dried.

[0186] Separately, 1 g of N,N'-methylene-bis-methacrylamide was dissolved in 20 ml of dimethylsulfoxide under stirring. To this solution, 20 g of methacrylamidopropyl-trimethylammonium chloride was added, and the total volume of the solution was adjusted to 80 ml by the addition of distilled water. Next, 0.5 g of azo-bis-amidinopropane was dissolved in 10 ml of water and then added to the solution of monomers. The latter was then adjusted to a total volume of 100 ml with water. Ninety milliliters of this solution were then added dropwise to the precoated POE-treated dry silica. The silica, impregnated with monomer solution, was then placed in a closed vessel at 80 °C and the polymerization was effected under nitrogen for two hours. The product so obtained was washed extensively with water and water-compatible solvents at acidic and alkaline pH values to eliminate any unpolymerized materials and reaction by products.

[0187] The cationic (i.e., anion-exchange) resin so obtained exhibited an ion-exchange capacity of 170 microequivalents/ml of quaternary ammonium groups and displayed a reversible BSA sorption capacity of 115 mg/ml. No non-specific binding was evident during a chromatographic separation conducted with the material.

EXAMPLE 24: Preparation of an Anion-Exchange Resin Using a Surface-Protected (i.e., Precoated) and PVP-Passivated Porous Silica Support

[0188] Polystyrene pellets (10 g, average molecular weight approximately 400 Ku (kD) are dissolved in 10 ml of methylene chloride and then added dropwise to 100 g of porous silica with characteristics described in the previous example. After about 30 minutes of shaking, the mixture is dried under an air stream at room temperature until total evaporation of the chlorinated solvent has occured, as evidenced by attainment of a constant particle weight. The dry powder is then heated overnight at 190-200 °C.

[0189] This polystyrene-coated silica is then suspended in 200 ml of an aqueous solution of 5% polyvinylpyrrolidone (PVP) with an average molecular weight of about 400 Ku (kD). The mixture is stirred gently for about 5 hours at

85°C and then the excess solution is removed by filtration. The silica beads are then washed extensively with water to remove the excess POE; the beads are finally rinsed twice with pure ethanol and dried.

[0190] Separately, 1 g of N,N'-methylene-bis-methacrylamide are dissolved in 20 ml of dimethylsulfoxide under stirring, To this solution, 20 g of methacrylamidopropyl-trimethylammonium chloride are added, and the total volume of the solution is adjusted to 80 ml by the addition of distilled water. Next, 0.5 g of azo-bid-amidinopropane are dissolved in 10 ml of water and then added to the solution of monomers. The latter is then adjusted to a total volume of 100 ml with water. Ninety milliliters of this solution are then added dropwise to the precoated POE-treated dry silica. The silica, impregnated with monomer solution, is then placed in a closed vessel at 80 °C and the polymerization is effected under nitrogen for two hours. The product so obtained is washed extensively with water and water-compatible solvents at acidic and alkaline pH values to eliminate any unpolymerized materials and reaction by products. The cationic (i.e., anion-exchange) resin so obtained exhibits an ion-exchange capacity of about 160 microequivalents/ml of quaternary ammonium groups and displays a reversible BSA sorption capacity of about 120 mg/ml. Little or no non-specific binding is evident during a chromatographic separation conducted with the material.

EXAMPLE 25: Preparation of a Cation-Exchange Resin Using a Surface-Protected (i.e., Precoated) and POE-Passivated Porous Silica Support

[0191] Polystyrene pellets (10 g, average molecular weight approximately 400 Ku (kD) are dissolved in 10 ml of methylene chloride and then added dropwise to 100 g of porous silica with the following characteristics: a particle diameter of 25 to 60 µm (microns), a pore diameter of 300 nm (3000 Angstroms), a surface area of 15 m^2/g surface area, and a porous volume of about 1 cm^3/g. After about 30 minutes of shaking, the mixture is dried under an air stream at room temperature until total evaporation of the chlorinated solvent has occured, as evidenced by attainment of a constant particle weight. The dry powder is then heated overnight at 190-200 °C.

[0192] This polystyrene-coated silica is then suspended in 200 ml of an aqueous solution of 5% polyoxyethylene and stirred gently for about 5 hours at 85 °C. The excess solution is removed by filtration. The silica beads are then washed extensively with water to remove the excess POE; the beads are finally rinsed twice with pure ethanol and dried.

[0193] Next, 1 g of N,N'-methylene-bis-methacrylamide, 1 g of methacrylamidopropyltrimethylammonium chloride, and 18 g of acrylamidomethyl-propane sulfonic acid sodium salt are dissolved in 90 ml of a solvent mixture comprised of 20 ml of dimethylsulfoxide, 60 ml of water, and 10 ml of ethanol. To this solution 10 ml of water containing 0.5 g of azo-bis-amidinopropane are added. The final mixture so obtained is then added dropwise to the "dry", polystyrene-protected silica. This silica, impregnated with monomer solution, is then placed in a closed vessel at 80 °C and the polymerization is effected under nitrogen for a period of at least 3 hours. The polyanionic product so obtained is then washed extensively as described in the immediately preceding examples.

[0194] The resin so obtained exhibits an ion-exchange capacity of about 100 microequivalents/ml of sulfonate groups and displays a reversible lysozyme sorption capacity of about 130 mg/ml.

Claims

1. A passivated porous support comprising (i) a porous mineral oxide or polymeric matrix having interior and exterior surfaces and innate groups that render said matrix susceptible to undesirable non-specific interaction with biological molecules, and (ii) a three-dimensional pore-filling polymer network derived from a passivation mixture comprising an optional main monomer, a neutralizing or passivating monomer different from said main monomer, and a crosslinking agent, said mixture having been allowed to come into intimate contact with said surfaces of said matrix such that on polymerization of said mixture said innate groups of said matrix become deactivated, resulting in the substantial elimination of said undesirable non-specific interaction.

2. A passivated porous support as claimed in claim 1 in which said matrix comprises silica and said innate groups are silanol groups.

3. A passivated porous support as claimed in claim 1 in which said matrix comprises alumina.

4. A passivated porous support as claimed in claims 1, 2 or 3 comprising (i) a porous mineral oxide matrix having interior and exterior surfaces substantially covered by a thin, protective polymer surface coating characterized by innate hydrophobic groups that render said coating susceptible to undesirable non-specific interaction with one or more biological molecules, and (ii) a three-dimensional pore-filling polymer network derived from a passivation mixture comprising an optional main monomer, a passivating monomer different from said main monomer, and a crosslinking agent, said mixture having been allowed to come into intimate contact with said surfaces of said coat-

ing such that on polymerization of said mixture said innate groups of said coating become deactivated, resulting in the substantial elimination of said undesirable non-specific interaction.

5. A passivated porous support as claimed in claim 1 in which said matrix comprises a polymeric material and said innate groups are hydrophobic groups.

6. A passivated porous support as claimed in claim 5, in which said polymeric material is polystyrene.

7. A passivated porous support as claimed in any one of the preceding claims which is further characterized by reversible high sorptive capacity.

8. A passivated porous support as claimed in any one of the preceding claims which is further characterized by chemical stability on exposure to a strong acidic or alkaline medium.

9. A passivated porous support as claimed in any one of the preceding claims which is further characterized by chemical stability on exposure to a strong oxidizing medium.

10. A passivated porous support as claimed in any one of the preceding claims in which said matrix has an initial average particle size ranging from 5 to 1000 µm (microns).

11. A passivated porous support as claimed in claim 10 in which said matrix has an initial average particle size ranging from 10 to 100 µm (microns).

12. A passivated porous support as claimed in any one of the preceding claims in which said matrix has an initial porous volume ranging from 0.2 to 2 cm^3/gram.

13. A passivated porous support as claimed in any one of the preceding claims in which said matrix has an initial surface area ranging from 1 to 800 m^2/gram.

14. A passivated porous support as claimed in any one of the preceding claims in which said matrix has an initial pore size ranging from 5 to 600 nm (50 to 6000 angstroms).

15. A passivated porous support as claimed in any one of the preceding claims which is further characterized as having a size exclusion limit ranging from 500 to 2,000,000 u (daltons).

16. A passivated porous support as claimed in claim 7 in which said reversible sorptive capacity for said biological molecule ranges from 1 to 300 milligrams per milliter of passivated porous support bed.

17. A passivated porous support as claimed in any one of the preceding claims in which said polymerization of said passivation mixture is effected in the presence of a pore inducer.

18. A passivated porous support as claimed in claim 17 in which said pore inducer is selected from the group consisting of polyethylene glycol, polyoxyethylene, and polysaccharide.

19. A passivated porous support as claimed in any one of the preceding claims in which said polymerization of said passivation mixture is effected in the presence of a polar solvent.

20. A passivated porous support as claimed in claim 19 in which said polar solvent is selected from the group consisting of an alcohol, a cyclic ether, a ketone, a tertiary amide, a dialkyl sulfoxide, and mixtures thereof.

21. A passivated porous support as claimed in claim 20 in which said polar solvent is selected from the group consisting of methanol, ethanol, propanol, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, acetone, dioxane, and mixtures thereof.

22. A passivated porous support as claimed in any one of the preceding claims in which said polymerization of said passivation mixture is effected in the presence of a polymerization initiator.

23. A passivated porous support as claimed in claim 22 in which said polymerization initiator is selected from the group

consisting of persulfate/tertiary amine, nitriles, transition metals, and photochemical initiators.

24. A passivated porous support as claimed in claims 22 or 23 in which said polymerization of said passivation mixture is effected by radiant energy.

25. A passivated porous support as claimed in any one of the preceding claims in which said optional main monomer comprises a vinyl monomer having at least one polar substituent.

26. A passivated porous support as claimed in claim 25 in which said polar substituent is nonionic.

27. A passivated porous support as claimed in claim 25 in which said polar substituent is ionic or ionizable.

28. A passivated porous support as claimed in claims 25, 26 or 27 in which said vinyl monomer has at least two polar substituents that may be ionic, nonionic, ionizable or a combination thereof.

29. A passivated porous support as claimed in any one of the preceding claims in which said optional main monomer is selected to provide a polymer network that has an affinity for a preselected biological molecule.

30. A passivated porous support as claimed in any one of the preceding claims in which said neutralizing monomer comprises a vinyl monomer having at least one polar ionic or ionizable substituent.

31. A passivated porous support as claimed in claim 30 in which said substituent is positively charged.

32. A passivated porous support as claimed in any one of the preceding claims in which said neutralizing monomer is selected to provide a polymer network that is effective to deactivate hydroxyl groups on the surfaces of said matrix.

33. A passivated porous support as claimed in any one of the preceding claims in which said neutralizing monomer is selected from the group consisting of diethylaminoethyl acrylamide, diethylaminoethyl methacrylamide, dimethylaminoethyl methacrylate, methacrylamido propyltrimethyl ammonium halide, triethylaminoethyl acrylamide, trimethylaminoethyl methacrylate, polyethyleneglycol dimethacrylate, and polyethyleneglycol diacrylate.

34. A passivated porous support as claimed in any one of the preceding claims in which said crosslinking agent comprises a vinyl monomer having at least one other polymerizable group.

35. A passivated porous support as claimed in claim 34 in which said polymerizable group is selected from the group consisting of a double bond, a triple bond, an allylic group, an epoxide, an azetidine, and a strained carbocyclic ring.

36. A passivated porous support as claimed in any one of the preceding claims in which said crosslinking agent is selected from group consisting of N,N'-methylenebis(acrylamide), N,N'-methylenebis(methacrylamide), diallyl tartradiamide, allyl methacrylate, diallyl amine, diallyl ether, diallyl carbonate, divinyl ether, 1,4-butanedioldivinylether, polyethyleneglycol divinyl ether, and 1,3-diallyloxy-2-propanol.

37. A passivated porous support as claimed in any one of the preceding claims in which said innate groups can participate in hydrogen bonding or electrostatic interactions.

38. A passivated porous support as claimed in claim 25 in which said polar substituent is positively charged.

39. A passivated porous support as claimed in claim 25 in which said polar substituent is negatively charged.

40. A passivated porous support as claimed in claim 4 in which said coating comprises a linear polymer capable of being dissolved in a suitable solvent to form a coating solution, said linear polymer being selected from the group consisting of polystyrene, polysulfone, polyethersulfone, cellulose acetate, cellulose nitrate, polyvinylacetate, polyacrylates, polyvinylidine fluoride, polyacrylonitrile, polyamides, and polyimides.

41. A passivated porous support as claimed in claim 40 in which said polymeric coating comprises polystyrene.

42. A passivated porous support as claimed in claim 23 in which said polymerization initiator is azo-bis-amidino-propane.

43. A passivated porous support as claimed in any one of the preceding claims in which said polymerization of said passivation mixture is effected by thermal energy.

44. A passivated porous support as claimed in claim 4 in which said passivating monomer comprises a vinyl monomer having at least one hydrophobic substituent.

45. A passivated porous support as claimed in claim 44 in which said hydrophobic substituent on said passivating monomer contains from 1 to 20 carbon atoms and is selected from the group consisting of straight-chain alkyl groups, branched-chain alkyl groups, aromatic groups, and arylaromatic groups.

46. A passivated porous support as claimed in claim 4 in which said passivating monomer is selected to deactivate hydrophobic groups on said surface coating.

47. A passivated porous support as claimed in claim 46 in which said passivating monomer is selected from the group consisting of N-alkylacrylamides, N-arylacrylamides, and derivatives thereof.

48. A passivated porous support as claimed in claim 47 in which said passivating monomer is selected from the group consisting of N-tert-octylacrylamide, N-(1-methylundecyl)-acrylamide, N-(1,1,3,5-tetramethyl)octylacrylamide, Triton-X-100-methacrylate, and polyethyleneglycol-dimethacrylate.

49. A passivated porous support as claimed in claim 4 in which said innate hydrophobic groups present in said polymer surface coating can participate in hydrophobic-hydrophobic bonding interactions with a hydrophobic substituent present in said passivating monomer.

50. A passivated porous support as claimed in claim 5 in which said matrix comprises a hydrophobic polymer selected from the group consisting of polystyrene, polysulfone, polyethersulfone, cellulose acetate, cellulose nitrate, polyethylene, polypropylene, polyvinylacetate, polyacrylates, polyvinylidine fluoride, polyacrylonitrile, polyamides, and polyimides.

51. A method of passivating a porous mineral oxide or polymeric matrix having interior and exterior surfaces and innate groups that render said matrix susceptible to undesirable non-specific interaction with biological molecules, comprising:

(a) contacting said surfaces of said matrix with a passivation mixture comprising effective amounts of an optional main monomer, a neutralizing or passivating monomer different from said main monomer, and a crosslinking agent;
(b) effecting the polymerization of said mixture to form a three-dimensional pore-filling polymer network within and substantially filling the pores of said matrix, such that said innate groups of said matrix become deactivated, resulting in the substantial elimination of said undesirable nonspecific interaction.

52. A method as claimed in claim 51, wherein said porous matrix is a mineral oxide matrix having interior and exterior surfaces substantially covered by a thin, protective polymer surface coating characterized by innate hydrophobic groups that render said coating susceptible to undesirable non-specific interaction with one or more biological molecules, said method comprising:

(a) contacting said surfaces of said polymer-coated matrix with a passivation mixture comprising effective amounts of an optional main monomer, a passivating monomer different from said main monomer, and a crosslinking agent;
(b) effecting the polymerization of said mixture to form a three-dimensional pore-filling polymer network within and substantially filling the pores of said polymer-coated matrix, such that said innate hydrophobic groups of said coating polymer become substantially covered and thereby deactivated, resulting in a significant reduction of the strength and extent of said undesirable non-specific interaction.

53. A method as claimed in claim 51, wherein the matrix comprises a polymeric material having interior and exterior surfaces and innate hydrophobic groups that render said matrix susceptible to undesirable non-specific interaction with one or more biological molecules, said method comprising:

(a) contacting said surfaces of said matrix with a passivation mixture comprising effective amounts of an

optional main monomer, a passivating monomer different from said main monomer, and a crosslinking agent;
(b) effecting the polymerization of said mixture to form a thee-dimensional pore-filling polymer network within and substantially filling the pores of said matrix, such that said innate hydrophobic groups of said matrix become substantially covered and thereby deactivated, resulting in a significant reduction of the strength and extent of said undesirable non-specific interaction.

54. A method as claimed in claims 51, 52 or 53 in which the amount of neutralizing or passivating monomer is sufficient to deactivate or cover the innate groups present on the surfaces of said matrix.

55. A method as claimed in any one of claims 51-54 in which said surfaces of said matrix are contacted with a solution of said passivation mixture.

56. A method as claimed in claim 55 in which the volume of a solution of said passivation mixture in ml is approximately equal to the weight of said matrix in grains.

57. A method as claimed in claim 55 in which the volume of a solution of said passivation mixture is approximately equal to the porous volume of said matrix.

58. A method as claimed in claims 55, 56 or 57 in which said solution is aqueous.

59. A method as claimed in any one of claims 51-58 in which said polymerization of said passivation mixture is effected in the presence of a pore inducer.

60. A method as claimed in any one of claims 51-59 in which said polymerization of said passivation mixture is effected in the presence of a polymerization initiator.

61. A method as claimed in any one of claims 51-60 in which said polymerization of said passivation mixture is effected by radiant or thermal energy.

62. A method of separating a preselected biological molecule from a sample containing the same comprising:

(a) loading a column packed with a passivated porous support as claimed in claim 29 with a sample containing a preselected biological molecule to be separated from a mixture;
(b) passing an eluent solution through said loaded column to effect the separation of said biological molecule.

63. A method as claimed in claim 62 in which said biological molecule is a protein.

64. A method as claimed in claim 62 in which said biological molecule is a carbohydrate.

65. A method as claimed in claim 62 in which said biological molecule is a polynucleotide.

66. A chromatographic method for the separation of biological molecules comprising passing a sample containing a mixture of biological molecules through a column packed with a passivated porous support as claimed in any one of claims 1-50.

67. A chromatographic method as claimed in claim 66 wherein said column is selected from the group consisting of a packed column and a fluidized-bed column.

Patentansprüche

1. Ein passivierter Träger umfassend (i) eine poröse Mineraloxid- oder Polymermatrix mit Innen- und Außenflächen und innewohnenden Gruppen, die besagte Matrix empfänglich für unerwünschte nichtspezifische Wechselwirkungen mit biologischen Molekülen machen, und (ii) ein dreidimensionales porenfüllendes Polymernetz, das hervorgegangen ist aus einem Passivierungsgemisch bestehend aus einem optionalen Hauptpolymer, einem neutralisierenden oder passivierenden und von besagtem Hauptpolymer abweichenden Monomer sowie einem Vernetzungsmittel, wobei es besagtem Gemisch gestattet wurde, in innigen Kontakt mit besagten Oberflächen der besagten Matrix zu treten, so daß nach der Polymerisation der besagten Gemische die innewohnenden Gruppen der besagten Matrix deaktiviert werden mit der Folge, daß besagte unerwünschte nichtspezifische Wechselwirkun-

gen im wesentlichen eliminiert werden.

2. Ein passivierter Träger entsprechend Anspruch 1, dadurch gekennzeichnet, daß besagte Matrix Siliciumdioxid enthält und besagte innewohnende Gruppen Silanolgruppen sind.

3. Ein passivierter Träger entsprechend Anspruch 1, dadurch gekennzeichnet, daß besagte Matrix Aluminiumoxid enthält.

4. Ein passivierter Träger entsprechend Anspruch 1, 2 oder 3, umfassend (i) eine poröse Mineraloxidmatrix mit Innen- und Außenflächen, die im wesentlichen mit einer dünnen Polymeroberflächenschutzbeschichtung versehen sind, die durch innewohnende hydrophobe Gruppen gekennzeichnet ist, die besagte Beschichtung empfänglich machen für unerwünschte nichtspezifische Wechselwirkungen mit ein oder mehreren biologischen Molekülen, und (ii) ein dreidimensionales porenfüllendes Polymernetz, das hervorgegangen ist aus einem Passivierungsgemisch bestehend aus einem optionalen Hauptpolymer, einem passivierenden und von besagtem Hauptpolymer abweichenden Monomer sowie einem Vernetzungsmittel, wobei es besagtem Gemisch gestattet wurde, in innigen Kontakt mit besagten Oberflächen der besagten Matrix zu treten, so daß nach der Polymerisation der besagten Gemische die innewohnenden Gruppen der besagten Matrix deaktiviert werden mit der Folge, daß besagte unerwünschte nichtspezifische Wechselwirkungen im wesentlichen eliminiert werden.

5. Ein passivierter Träger entsprechend Anspruch 1, dadurch gekennzeichnet, daß besagte Matrix ein Polymermaterial enthält und besagte innewohnende Gruppen hydrophobe Gruppen sind.

6. Ein passivierter Träger entsprechend Anspruch 5, dadurch gekennzeichnet, daß es sich bei besagtem Polymermaterial um Polystyren handelt.

7. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, desweiteren gekennzeichnet durch eine umkehrbare hohe Sorptionsfähigkeit.

8. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, desweiteren gekennzeichnet durch chemische Beständigkeit gegenüber dem Einfluß eines stark sauren oder alkalischen Mittels.

9. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, desweiteren gekennzeichnet durch chemische Beständigkeit gegenüber dem Einfluß eines starken Oxidationsmittels.

10. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Matrix eine anfängliche durchschnittliche Partikelgröße aufweist, die sich zwischen 5 und 1000 µm bewegt.

11. Ein passivierter Träger entsprechend Anspruch 10, dadurch gekennzeichnet, daß besagte Matrix eine anfängliche durchschnittliche Partikelgröße aufweist, die sich zwischen 10 und 100 µm bewegt.

12. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet daß besagte Matrix ein anfängliches durchschnittliches Porenvolumen aufweist, das sich zwischen 0,2 bis 2 cm^3/g bewegt.

13. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Matrix eine anfängliche Oberfläche aufweist, die sich zwischen 1 und 800 m^2/g bewegt.

14. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Matrix eine anfängliche Porengröße aufweist, die sich zwischen 5 und 600 nm (50 bis 6000 Angström) bewegt.

15. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, desweiteren dadurch gekennzeichnet, daß er eine Größenausschließungsgrenze in dem Bereich von 600 bis 2,000,000 µ (Dalton) aufweist.

16. Ein passivierter Träger entsprechend Anspruch 7, dadurch gekennzeichnet, daß besagte umkehrbare Sorptionsfähigkeit für besagte biologische Moleküle in dem Bereich zwischen 1 und 300 Milligramm pro Milliliter des passivierten porösen Trägerbetts liegt.

17. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Passivierungsgemischs in der Gegenwart eines Poreninduzierers veranlaßt wird.

18. Ein passivierter Träger entsprechend Anspruch 17, dadurch gekennzeichnet, daß besagter Poreninduzierer ausgewählt wird aus der Gruppe umfassend Polyethylenglycol, Polyoxyethylen und Polysaccharid.

19. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Passivierungsgemischs in der Gegenwart eines polaren Lösemittels veranlaßt wird.

20. Ein passivierter Träger entsprechend Anspruch 19, dadurch gekennzeichnet, daß besagtes polares Lösemittel ausgewählt wird aus der Gruppe umfassend einen Alkohol, einen zyklischen Ether, ein Keton, ein tertiäres Amid, ein Dialkylsulfoxid und aus diesen gebildete Gemische.

21. Ein passivierter Träger entsprechend Anspruch 20, dadurch gekennzeichnet, daß besagtes polares Lösemittel ausgewählt wird aus der Gruppe umfassend Methanol, Ethanol, Propanol, Tetrahydrofuran, Dimethylsulfoxid, Dimethylformamid, Aceton, Dioxan und aus diesen gebildete Gemische.

22. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Passivierungsgemischs in der Gegenwart eines Polymerisationsinitiators veranlaßt wird.

23. Ein passivierter Träger entsprechend Anspruch 22, dadurch gekennzeichnet, daß besagter Polymerisationsinitiator ausgewählt wird aus der Gruppe umfassend Persulfat/tertiäres Amin, Nitrile, Übergangsmetalle und photochemische initiatoren.

24. Ein passivierter Träger entsprechend Anspruch 22 oder 23, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Passivierungsgemischs durch Strahlungsenergie veranlaßt wird.

25. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, das besagtes optionales Hauptmonomer ein Vinylmonomer enthält, das mindestens einen polaren Substituenten aufweist,

26. Ein passivierter Träger entsprechend Anspruch 25, dadurch gekennzeichnet, daß besagter polarer Substituent nichtionisch ist.

27. Ein passivierter Träger entsprechend Anspruch 25, dadurch gekennzeichnet, daß besagter polarer Substituent ionisch oder ionisierbar ist.

28. Ein passivierter Träger entsprechend Anspruch 25, 26 oder 27, dadurch gekennzeichnet, daß besagtes Vinylmonomer mindestens zwei polare Substituenten aufweist die ionisch, nichtionisch, oder ionisierbar sein können oder eine Kombination dieser Eigenschaften aufweisen können.

29. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagtes optionales Hauptmonomer so ausgewählt wird, daß ein Polymernetz gebildet wird, das eine Affinität zu einem vorabgewählten biologischen Molekül besitzt.

30. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagtes neutralisierendes Monomer ein Vinylmonomer enthält, das mindestens einen polaren ionischen oder ionisierbaren Substituenten aufweist.

31. Ein passivierter Träger entsprechend Anspruch 30, dadurch gekennzeichnet, daß besagter Substituent positiv geladen ist.

32. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagtes neutralisierendes Monomer so ausgewählt wird, daß ein Polymernetz gebildet wird, das bewirkt, daß Hydroxylgruppen auf den Oberflächen der besagten Matrix deaktiviert werden.

33. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagtes neutralisierendes Monomer ausgewählt wird aus der Gruppe umfassend Diethylaminoethylacrylamid, Diethylaminoethyl-methacrylamid, Dimethylaminoethylmethacrylat, Methacrylamid-propyltrimethyl-ammoniumhalogenid, Triethylaminoethyl-acrylamid, Trimethylaminoethyl-acrylamid, Trimethylaminoethyl-methacrylat, Polyethylenglycol-dimethacrytat und Polyethylenglycol-diacrylat.

34. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet daß besagtes Vernetzungsmittel ein Vinylmonomer enthält, das mindestens eine andere polymerisierbare Gruppe aufweist

35. Ein passivierter Täger entsprechend Anspruch 34, dadurch gekennzeichnet, daß besagte polymerisierbere Gruppe ausgewählt wird aus der Gruppe umfassend eine Doppelbindung, eine Dreifachbindung, eine Allylgruppe, ein Epoxid, ein Azetidin sowie einen gespannten carbocyclischen Ring.

36. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagtes Vernetzungsmittel ausgewählt wird aus dar Gruppe umfassend N,N'-Methylenbis(acrylamid), N,N'-Methylenbis(methacrylamid), Diallyltartradiamid, Allylmethacrylat, Diallylamin, Diallylether, Diallylcarbonat, Divinylether, 1,4-Butandioldivinylether, Polyethylenglycoldivinylether und 1,3-Diallyloxy-2-propanol.

37. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet daß besagte innewohnende Gruppen an Wasserstoffbrückenbindungen oder elektrostatischen Wechselwirkungen beteiligt sein können.

38. Ein passivierter Träger entsprechend Anspruch 25, dadurch gekennzeichnet, daß besagter polarer Substituent positiv geladen ist.

39. Ein passivierter Träger entsprechend Anspruch 25, dadurch gekennzeichnet, daß besagter polarer Substituent negativ geladen ist.

40. Ein passivierter Träger entsprechend Anspruch 4, dadurch gekennzeichnet, daß besagte Beschichtung ein lineares Polymer aufweist, das sich in einem geeigneten Lösemittel lösen läßt, um eine Beschichtungslösung zu ergeben, wobei besagtes lineares Polymer ausgewählt wird aus der Gruppe umfassend Polystyren, Polysulfon, Polyethersulfon, Celluloseacetat, Cellulosenitrat, Polyvinylacetat, Polyacrylate, Polyvinylidinfluorid, Polyacrylnitril, Polyamide und Polyimide.

41. Ein passivierter Träger entsprechend Anspruch 40, dadurch gekennzeichnet, daß besagte Polymerbeschichtung Polystyren enthält.

42. Ein passivierter Träger entsprechend Anspruch 23, dadurch gekennzeichnet, daß es sich bei besagtem Polymerisationsinitiator um Azo-bis-amidino-propan handelt.

43. Ein passivierter Träger entsprechend den vorstehenden Ansprüchen, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Polymerisationsgemischs durch Wärmeenergie veranlaßt wird.

44. Ein passivierter Träger entsprechend Anspruch 4, dadurch gekennzeichnet, daß es sich bei besagtem passivierenden Monomer um ein Vinylmonomer handelt, das mindestens einen hydrophoben Substituenten aufweist.

45. Ein passivierter Träger entsprechend Anspruch 44, dadurch gekennzeichnet, daß besagter hydrophober Substituent in besagtem passivierenden Monomer zwischen 1 und 20 Kohlenstoffatome enthält und ausgewählt wird aus der Gruppe umfassend geradkettige Alkylgruppen, verzweigtkettige Alkylgruppen, aromatische Gruppen und arylaromatische Gruppen.

46. Ein passivierter Träger entsprechend Anspruch 4, dadurch gekennzeichnet, daß besagtes passivierendes Monomer im Hinblick darauf ausgewählt wird, hydrophobe Gruppen auf besagter Oberflächenbeschichtung zu deaktivieren.

47. Ein passivierter Träger entsprechend Anspruch 46, dadurch gekennzeichnet, daß besagtes passivierendes Monomer ausgewählt wird aus der Gruppe umfassend N-Alkylacrylamide, N-Arylacrylamide und Derivate derselben.

48. Ein passivierter Träger entsprechend Anspruch 47, dadurch gekennzeichnet, daß besagtes passivierendes Monomer ausgewählt wird aus der Gruppe umfassend N-tert-Octylacrylamid, N-(1-Methylundecyl)-acrylamid, N-(1,1,3,5-Tetramethyl)octylacrylamid, Triton-X-100-Methacrylat und Polyethylenglycol-dimethacrylat.

49. Ein passivierter Träger entsprechend Anspruch 4, dadurch gekennzeichnet, daß die in besagter Polymeroberflächenbeschichtung befindlichen innenwohnenden hydrophoben Gruppen an hydrophob-hydrophob-Bindungs-Inter-

aktionen mit einem hydrophoben Substituenten, der in besagtem passivierenden Monomer vorkommt, mitwirken können.

50. Ein passivierter Träger entsprechend Anspruch 5, dadurch gekennzeichnet, daß besagte Matrix ein hydrophobes Polymer enthält, das ausgewählt wird aus der Gruppe umfassend Polystyren, Polysulfon, Polyethersulfon, Celluloseacetat, Cellulosenitrat, Polyethylen, Polypropylen, Polyvinylacetat, Polyacrylate, Polyvinylidinfluorid, Polyacrylnitril, Polyamide und Polyimide.

51. Ein Verfahren zur Passivierung einer porösen Mineraloxid- oder Polymermatrix mit Innen- und Außenflächen und innewohnenden Gruppen, die besagte Matrix empfänglich machen für unerwünschte nichtspezifische Wechselwirkungen mit biologischen Molekülen, die nachgenannten Verfahrensschritte umfassend:

(a) Inkontaktbringen der besagten Oberflächen der besagten Matrix mit einem Passivierungsgemisch bestehend aus wirksamen Mengen eines optionalen Hauptmonomers, eines neutralisierenden oder passivierenden und von besagtem Hauptmonomer abweichenden Monomers sowie eines Vernetzungsmittels;
(b) Veranlassung der Polymerisation des besagten Gemischs, um ein dreidimensionales porenfüllenden Polymernetzwerk innerhalb der Matrix hervorzubringen, das die Poren dieser Matrix im wesentlichen ausfüllt, so daß besagter innewohnende Gruppen der besagten Matrix deaktiviert werden mit der Folge, daß besagte unerwünschte nichtspezifische Wechselwirkungen im wesentlichen eliminiert werden.

52. Ein Verfahren entsprechend Anspruch 51, wobei besagte poröse Matrix eine Mineraloxidmatrix mit Innen- und Außenflächen ist, die im wesentlichen mit einer dünnen Polymeroberflächenschutzbeschichtung versehen sind, die durch innewohnende hydrophobe Gruppen gekennzeichnet ist, die besagte Beschichtung empfänglich machen für unerwünschte nichtspezifische Wechselwirkungen mit ein oder mehreren biologischen Molekülen, die nachgenannten Verfahrensschritte umfassend:

(a) Inkontaktbringen der besagten Oberflächen der besagten polymerbeschichteten Matrix mit einem Passivierungsgemisch bestehend aus wirksamen Mengen eines optionalen Hauptmonomers, eines passivierenden und von besagtem Hauptmonomer abweichenden Monomers sowie eines Vernetzungsmittels;
(b) Veranlassung der Polymerisation des besagten Gemischs, um ein dreidimensionales porenfühlenden Polymernetzwerk innerhalb der besagten polymerbeschichteten Matrix hervorzubringen, das die Poren dieser Matrix im wesentlichen ausfüllt, so daß besagte innewohnende hydrophobe Gruppen des besagten Beschichtungspolymers im wesentlichen überdeckt und dadurch deaktiviert werden mit der Folge, daß Stärke und Ausmaß der besagten unerwünschten nichtspezifischen Wechselwirkungen bedeutend reduziert werden.

53. Ein Verfahren entsprechend Anspruch 51, wobei die Matrix aus einem Polymermaterial mit Innen- und Außenflächen und innewohnenden hydrophoben Gruppen besteht, die besagte Matrix empfänglich machen für unerwünschte nichtspezifische Wechselwirkungen mit ein oder mehreren biologischen Molekülen, die nachgenannten Verfahrensschritte umfassend:

(a) Inkontaktbringen der besagten Oberflächen der besagten Matrix mit einem Passivierungsgemisch bestehend aus wirksamen Mengen eines optionalen Hauptmonomers, eines passivierenden und von besagtem Hauptmonomer abweichenden Monomers sowie eines Vernetzungsmittels;
(b) Veranlassung der Polymerisation des besagten Gemischs, um ein dreidimensionales porenfüllenden Polymernetzwerk innerhalb des besagten polymerbeschichteten Matrix hervorzubringen, das die Poren dieser Matrix im wesentlichen ausfüllt, so daß besagte innewohnende hydrophobe Gruppen des besagten Beschichtungspolymers im wesentlichen überdeckt und dadurch deaktiviert weiden mit der Folge, daß Stärke und Ausmaß der besagten unerwünschten nichtspezifischen Wechselwirkungen bedeutend reduziert werden.

54. Ein Verfahren entsprechend Anspruch 51, 52 oder 53, dadurch gekennzeichnet, daß die Menge des neutralisierenden oder passivierenden Monomers ausreicht, um die an den Oberflächen der besagten Matrix befindlichen innewohnenden Gruppen zu deaktivieren oder zu überdecken.

55. Ein Verfahren entsprechend den Ansprüchen 51 bis 54, dadurch gekennzeichnet, daß besagte Oberflächen der besagten Matrix mit einer Lösung des besagten Passivierungsgemischs in Kontakt gebracht werden.

56. Ein Verfahren entsprechend Anspruch 55, dadurch gekennzeichnet, daß das Volumen einer Lösung des besagten Passivierungsgemischs in Milliliter in etwa dem Gewicht der besagten Matrix in Gramm entspricht.

57. Ein Verfahren entsprechend Anspruch 55, dadurch gekennzeichnet, daß das Volumen einer Lösung des besagten Passivierungsgemischs in etwa dem Porenvolumen der besagten Matrix entspricht.

58. Ein Verfahren entsprechend Anspruch 55, 56 oder 57, dadurch gekennzeichnet, daß besagte Lösung wäßrig ist.

59. Ein Verfahren entsprechend den Ansprüchen 51 bis 58, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Passivierungsgemischs in der Gegenwart eines Poreninduzierers veranlaßt wird.

60. Ein Verfahren entsprechend den Ansprüchen 51 bis 59, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Polymerisationsgemischs in der Gegenwart eines Polymerisationsinitiators veranlaßt wird.

61. Ein Verfahren entsprechend den Ansprüchen 51 bis 60, dadurch gekennzeichnet, daß besagte Polymerisation des besagten Polymerisationsgemischs durch Strahlungs- oder Wärmeenergie veranlaßt wird.

62. Ein Verfahren zur Abtrennung eines vorabgewählten biologischen Moleküls von einer Probe, die nachgenannten Verfahrensschritte umfaßt:

(a) Beschicken einer mit einem passivierten porösen Träger entsprechend Anspruch 29 gefüllten Säule mit einer Probe, die ein vorabgewähltes biologisches Molekül enthält, um von einem Gemisch abgetrennt zu werden.
(b) Durchfließenlassen eines Elutionsmittels durch besagte geladene Säule, um die Abtrennung des besagten biologischen Moleküls zu veranlassen.

63. Ein Verfahren entsprechend Anspruch 62, dadurch gekennzeichnet, daß es sich bei besagtem biologischen Molekül um ein Protein handelt.

64. Ein Verfahren entsprechend Anspruch 62, dadurch gekennzeichnet, daß es sich bei besagtem biologischen Molekül um ein Kohlenhydrat handelt.

65. Ein Verfahren entsprechend Anspruch 62, dadurch gekennzeichnet, daß es sich bei besagtem biologischen Molekül um ein Polynucleotid handelt.

66. Ein chromatographisches Verfahren zur Abtrennung von biologischen Molekülen, umfassend das Durchfließenlassen einer ein Gemisch aus biologischen Molekülen enthaltenden Probe durch eine mit einem passivierten porösen Träger gefüllten Säule entsprechend den Ansprüchen 1 bis 50.

67. Ein chromatographisches Verfahren entsprechend Anspruch 66, dadurch gekennzeichnet, daß besagte Säule ausgewählt wird aus der Gruppe umfassend Füllkörpersäulen und Fließbettsäulen.

Revendications

1. Support poreux passivé comprenant (i) une matrice d'oxyde minéral ou polymère poreuse possédant des surfaces intérieure et extérieure et des groupes inhérents qui rendent ladite matrice sensible à une interaction non spécifique indésirable avec des molécules biologiques, et (ii) un réseau tridimensionnel de polymère qui remplit les pores, dérivé d'un mélange de passivation comprenant un monomère principal facultatif, un monomère de neutralisation ou de passivation différent dudit monomère principal, et un agent de réticulation, ledit mélange ayant été amené à entrer en contact intime avec lesdites surfaces de ladite matrice de façon que, lors de la polymérisation dudit mélange, lesdits groupes inhérents de ladite matrice soient désactivés, avec pour résultat une élimination substantielle de ladite interaction non spécifique indésirable.

2. Support poreux passivé selon la revendication 1, dans lequel ladite matrice comprend de la silice et lesdits groupes inhérents sont des groupes silanol.

3. Support poreux passivé selon la revendication 1, dans lequel ladite matrice comprend de l'alumine.

4. Support poreux passivé selon les revendications 1, 2 ou 3 comprenant (i) une matrice d'oxyde minéral poreuse possédant des surfaces intérieure et extérieure essentiellement recouvertes d'un revêtement superficiel protecteur, mince, à base d'un polymère, caractérisé par des groupes hydrophobes inhérents qui rendent ledit revête-

ment sensible à une interaction non spécifique indésirable avec une ou plusieurs molécules biologiques, et (ii) un réseau tridimensionnel de polymère qui remplit les pores, dérivé d'un mélange de passivation comprenant un monomère principal facultatif, un monomère de passivation différent dudit monomère principal, et un agent de réticulation, ledit mélange ayant été amené à entrer en contact intime avec lesdites surfaces dudit revêtement de façon que, lors de la polymérisation dudit mélange, lesdits groupes inhérents dudit revêtement soient désactivés, avec pour résultat une élimination substantielle de ladite interaction non spécifique indésirable.

5. Support poreux passivé selon la revendication 1, dans lequel ladite matrice comprend un matériau polymère et lesdits groupes inhérents sont des groupes hydrophobes.

6. Support poreux passivé selon la revendication 5, dans lequel ledit matériau polymère est le polystyrène.

7. Support poreux passivé selon l'une quelconque des revendications précédentes qui est, en outre, caractérisé par une capacité de sorption élevée, réversible.

8. Support poreux passivé selon l'une quelconque des revendications précédentes qui est, en outre, caractérisé par une stabilité chimique lorsqu'il est exposé à un milieu fortement acide ou alcalin.

9. Support poreux passivé selon l'une quelconque des revendications précédentes qui est, en outre, caractérisé par une stabilité chimique lorsqu'il est exposé à un milieu fortement oxydant.

10. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite matrice a une taille de particules moyenne initiale allant de 5 à 1000 µm (microns).

11. Support poreux passivé selon la revendication 10, dans lequel ladite matrice a une taille de particules moyenne initiale allant de 10 à 100 µm (microns).

12. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite matrice a un volume de pores initial allant de 0,2 à 2 cm^3/gramme.

13. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite matrice a une aire spécifique initiale allant de 1 à 800 m^2/gramme.

14. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite matrice a une taille de pores initiale allant de 5 à 600 mn (50 à 6000 angströms).

15. Support poreux passivé selon l'une quelconque des revendications précédentes qui est, en outre, caractérisé par une limite d'exclusion allant de 500 à 2 000 000 u (daltons).

16. Support poreux passivé selon la revendication 7, dans lequel ladite capacité de sorption réversible pour ladite molécule biologique va de 1 à 300 milligrammes par millilitre de lit de support poreux passivé.

17. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite polymérisation dudit mélange de passivation est mise en oeuvre en présence d'un inducteur de pores.

18. Support poreux passivé selon la revendication 17, dans lequel ledit inducteur de pores est choisi dans le groupe comprenant le polyéthylène glycol, le polyoxyéthylène, et un polysaccharide.

19. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite polymérisation dudit mélange de passivation est mise en oeuvre en présence d'un solvant polaire.

20. Support poreux passivé selon la revendication 19, dans lequel ledit solvant polaire est choisi dans le groupe comprenant un alcool, un éther cyclique, une cétone, un amide tertiaire, un dialkylsulfoxyde, et leurs mélanges.

21. Support poreux passivé selon la revendication 20, dans lequel ledit solvant polaire est choisi dans le groupe comprenant le méthanol, l'éthanol, le propanol, le tétrahydrofurane, le diméthylsulfoxyde, le diméthylformamide, l'acétone, le dioxanne, et leurs mélanges.

22. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite polymérisation dudit mélange de passivation est mise en oeuvre en présence d'une amorce de polymérisation.

23. Support poreux passivé selon la revendication 22, dans lequel ladite amorce de polymérisation est choisie dans le groupe comprenant le persulfate/amine tertiaire, les nitriles, les métaux de transition, et les amorces photochimiques.

24. Support poreux passivé selon les revendications 22 ou 23, dans lequel ladite polymérisation dudit mélange de passivation est induite par une énergie rayonnante.

25. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit monomère principal facultatif comprend un monomère de vinyle possédant au moins un substituant polaire.

26. Support poreux passivé selon la revendication 25, dans lequel ledit substituant polaire est non ionique.

27. Support poreux passivé selon la revendication 25, dans lequel ledit substituant polaire est ionique ou ionisable.

28. Support poreux passivé selon les revendications 25, 26 ou 27, dans lequel ledit monomère de vinyle possède au moins deux substituants polaires qui peuvent être ioniques, non ioniques, ionisables ou une combinaison de ceux-ci.

29. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit monomère principal facultatif est choisi de manière à former un réseau de polymère qui possède une affinité pour une molécule biologique présélectionnée.

30. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit monomère de neutralisation comprend un monomère de vinyle possédant au moins un substituant polaire ionique ou ionisable.

31. Support poreux passivé selon la revendication 30, dans lequel ledit substituant est chargé positivement.

32. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit monomère de neutralisation est choisi de manière à former un réseau de polymère qui est efficace pour désactiver les groupes hydroxyle sur les surfaces de ladite matrice.

33. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit monomère de neutralisation est choisi dans le groupe comprenant le diéthylaminoéthylacrylamide, le diéthylaminoéthylméthacrylamide, le méthacrylate de diméthylaminoéthyle, l'halogénure de méthacrylamidopropyltriméthylammonium, le triéthylaminoéthylacrylamide, le méthacrylate de triméthylaminoéthyle, le diméthacrylate de polyéthylène glycol, et le diacrylate de polyéthyléne glycol.

34. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit agent de réticulation comprend un monomère de vinyle possédant au moins un autre groupe polymérisable.

35. Support poreux passivé selon la revendication 34, dans lequel ledit groupe polymérisable est choisi dans le groupe comprenant une double liaison, une triple liaison, un groupe allylique, un époxyde, une azétidine, et un cycle carbocyclique contraint.

36. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ledit agent de réticulation est choisi dans le groupe comprenant le N,N'-méthylènebis(acrylamide), le N,N'-méthylènebis (méthacrylamide), le tartradiamide de diallyle, le méthacrylate d'allyle, la diallylamine, l'éther diallylique, le carbonate de diallyle, l'éther divinylique, l'éther 1,4-butanediol-divinylique, l'éther polyéthylène glycol divinylique, et le 1,3-diallyloxy-2-propanol.

37. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel lesdits groupes inhérents peuvent participer à des interactions de liaison à l'hydrogène ou électrostatiques.

38. Support poreux passivé selon la revendication 25, dans lequel ledit substituant polaire est chargé positivement.

39. Support poreux passivé selon la revendication 25, dans lequel ledit substituant polaire est chargé négativement.

40. Support poreux passivé selon la revendication 4, dans lequel ledit revêtement comprend un polymère linéaire qui peut être dissous dans un solvant convenable de manière à former une solution de revêtement, ledit polymère linéaire étant choisi dans le groupe comprenant le polystyrène, la polysulfone, la polyéthersulfone, l'acétocellulose, la nitrocellulose, le polyacétate de vinyle, les polyacrylates, le polyfluorure de vinylidine, le polyacrylonitrile, les polyamides, et les polyimides.

41. Support poreux passivé selon la revendication 40, dans lequel ledit revêtement polymère comprend du polystyrène.

42. Support poreux passivé selon la revendication 23, dans lequel ladite amorce de polymérisation est l'azo-bis-amidino-propane.

43. Support poreux passivé selon l'une quelconque des revendications précédentes, dans lequel ladite polymérisation dudit mélange de passivation est induite par une énergie thermique.

44. Support poreux passivé selon la revendication 4, dans lequel ledit monomère de passivation comprend un monomère de vinyle possédant au moins un substituant hydrophobe.

45. Support poreux passivé selon la revendication 44, dans lequel ledit substituant hydrophobe sur ledit monomère de passivation contient 1 à 20 atomes de carbone et est choisi dans le groupe comprenant les groupes alkyle à chaîne droite, les groupes alkyle à chaîne ramifiée, les groupes aromatiques, et les groupes arylaromatiques.

46. Support poreux passivé selon la revendication 4, dans lequel ledit monomère de passivation est choisi pour désactiver les groupes hydrophobes sur ledit revêtement superficiel.

47. Support poreux passivé selon la revendication 46, dans lequel ledit monomère de passivation est choisi dans le groupe comprenant les N-alkylacrylamides, les N-arylacrylamides, et leurs dérivés.

48. Support poreux passivé selon la revendication 47, dans lequel ledit monomère de passivation est choisi dans le groupe comprenant le N-tert-octylacrylamide, le N-(1-méthylundécyl)acrylamide, le N-(1,1,3,5-tétraméthyl) octylacrylamide, le méthacrylate de Triton-X-100, et le diméthacrylate de polyéthylène glycol.

49. Support poreux passivé selon la revendication 4, dans lequel lesdits groupes hydrophobes inhérents présents dans ledit revêtement superficiel à base d'un polymère peuvent participer à des interactions de liaisons hydrophobes-hydrophobes avec un substituant hydrophobe présent dans ledit monomère de passivation.

50. Support poreux passivé selon la revendication 5, dans lequel ladite matrice comprend un polymère hydrophobe choisi dans le groupe comprenant le polystyrène, la polysulfone, la polyéthersulfone, l'acétocellulose, la nitrocellulose, le polyéthylène, le polypropylène, le polyacétate de vinyle, les polyacrylates, le polyfluorure de vinylidine, le polyacrylonitrile, les polyamides, et les polyimides.

51. Procédé de passivation d'une matrice d'oxyde minéral ou polymère poreuse possédant des surfaces intérieure et extérieure et des groupes inhérents qui rendent ladite matrice sensible à une interaction non spécifique indésirable avec des molécules biologiques, comprenant les étapes consistant à :

(a) mettre lesdites surfaces de ladite matrice en contact avec un mélange de passivation comprenant des quantités efficaces d'un monomère principal facultatif, d'un monomère de neutralisation ou de passivation différent dudit monomère principal, et d'un agent de réticulation ;
(b) mettre en oeuvre la polymérisation dudit mélange de manière à former un réseau tridimensionnel de polymère qui remplit les pores présents dans, et remplir essentiellement les pores de ladite matrice, de façon que lesdits groupes inhérents de ladite matrice soient désactivés, avec pour résultat une élimination substantielle de ladite interaction non spécifique indésirable.

52. Procédé selon la revendication 51, dans lequel ladite matrice poreuse est une matrice d'oxyde minéral possédant des surfaces intérieure et extérieure essentiellement recouvertes d'un revêtement superficiel protecteur, mince, à base d'un polymère, caractérisé par des groupes hydrophobes inhérents qui rendent ledit revêtement sensible à

une interaction non spécifique indésirable avec une ou plusieurs molécules biologiques, ledit procédé comprenant les étapes consistant à :

(a) mettre lesdites surfaces de ladite matrice revêtue d'un polymère en contact avec un mélange de passivation comprenant des quantités efficaces d'un monomère principal facultatif, d'un monomère de passivation différent dudit monomère principal, et d'un agent de réticulation ;
(b) mettre en oeuvre la polymérisation dudit mélange de manière à former un réseau tridimensionnel de polymère qui remplit les pores présents dans, et remplir essentiellement les pores de ladite matrice revêtue d'un polymère, de façon que lesdits groupes hydrophobes inhérents dudit polymère de revêtement soient substantiellement recouverts et, ainsi, désactivés, avec pour résultat une réduction significative de la force et de l'étendue de ladite interaction non spécifique indésirable.

53. Procédé selon la revendication 51, dans lequel la matrice comprend un matériau polymère possédant des surfaces intérieure et extérieure et des groupes hydrophobes inhérents qui rendent ladite matrice sensible à une interaction non spécifique indésirable avec une ou plusieurs molécules biologiques, ledit procédé comprenant les étapes consistant à :

(a) mettre lesdites surfaces de ladite matrice en contact avec un mélange de passivation comprenant des quantités efficaces d'un monomère principal facultatif, d'un monomère de passivation différent dudit monomère principal, et d'un agent de réticulation ;
(b) mettre en oeuvre la polymérisation dudit mélange de manière à former un réseau tridimensionnel de polymère qui remplit les pores présents dans, et remplir essentiellement les pores de ladite matrice, de façon que lesdits groupes hydrophobes inhérents de ladite matrice soient substantiellement recouverts et, ainsi, désactivés, avec pour résultat une réduction significative de la force et de l'étendue de ladite interaction non spécifique indésirable.

54. Procédé selon les revendications 51, 52 ou 53, dans lequel la quantité du monomère de neutralisation ou de passivation est suffisante pour désactiver ou recouvrir les groupes inhérents présents sur les surfaces de ladite matrice.

55. Procédé selon l'une quelconque des revendications 51 à 54, dans lequel lesdites surfaces de ladite matrice sont mises en contact avec une solution dudit mélange de passivation.

56. Procédé selon la revendication 55, dans lequel le volume d'une solution dudit mélange de passivation, en ml, est à peu près égal au poids de ladite matrice, en grammes.

57. Procédé selon la revendication 55, dans lequel le volume d'une solution dudit mélange de passivation est à peu près égal au volume de pores de ladite matrice.

58. Procédé selon les revendications 55, 56 ou 57, dans lequel ladite solution est aqueuse.

59. Procédé selon l'une quelconque des revendications 51 à 58, dans lequel ladite polymérisation dudit mélange de passivation est mise en oeuvre en présence d'un inducteur de pores.

60. Procédé selon l'une quelconque des revendications 51 à 59, dans lequel ladite polymérisation dudit mélange de passivation est mise en oeuvre en présence d'une amorce de polymérisation.

61. Procédé selon l'une quelconque des revendications 51 à 60, dans lequel ladite polymérisation dudit mélange de passivation est induite par une énergie rayonnante ou thermique.

62. Procédé de séparation d'une molécule biologique présélectionnée à partir d'un échantillon la contenant, qui comprend les étapes consistant à :

(a) charger une colonne garnie d'un support poreux passivé selon la revendication 29 avec un échantillon contenant une molécule biologique présélectionnée qui doit être séparée d'un mélange ;
(b) faire passer une solution d'éluant sur ladite colonne chargée pour induire la séparation de ladite molécule biologique.

63. Procédé selon la revendication 62, dans lequel ladite molécule biologique est une protéine.

64. Procédé selon la revendication 62, dans lequel ladite molécule biologique est un glucide.

65. Procédé selon la revendication 62, dans lequel ladite molécule biologique est un polynucléotide.

66. Procédé chromatographique de séparation de molécules biologiques consistant à faire passer un échantillon contenant un mélange de molécules biologiques sur une colonne garnie d'un support poreux passivé selon l'une quelconque des revendications 1 à 50.

67. Procédé chromatographique selon la revendication 66, dans lequel ladite colonne est choisie dans le groupe comprenant une colonne garnie ou une colonne à lit fluidisé.

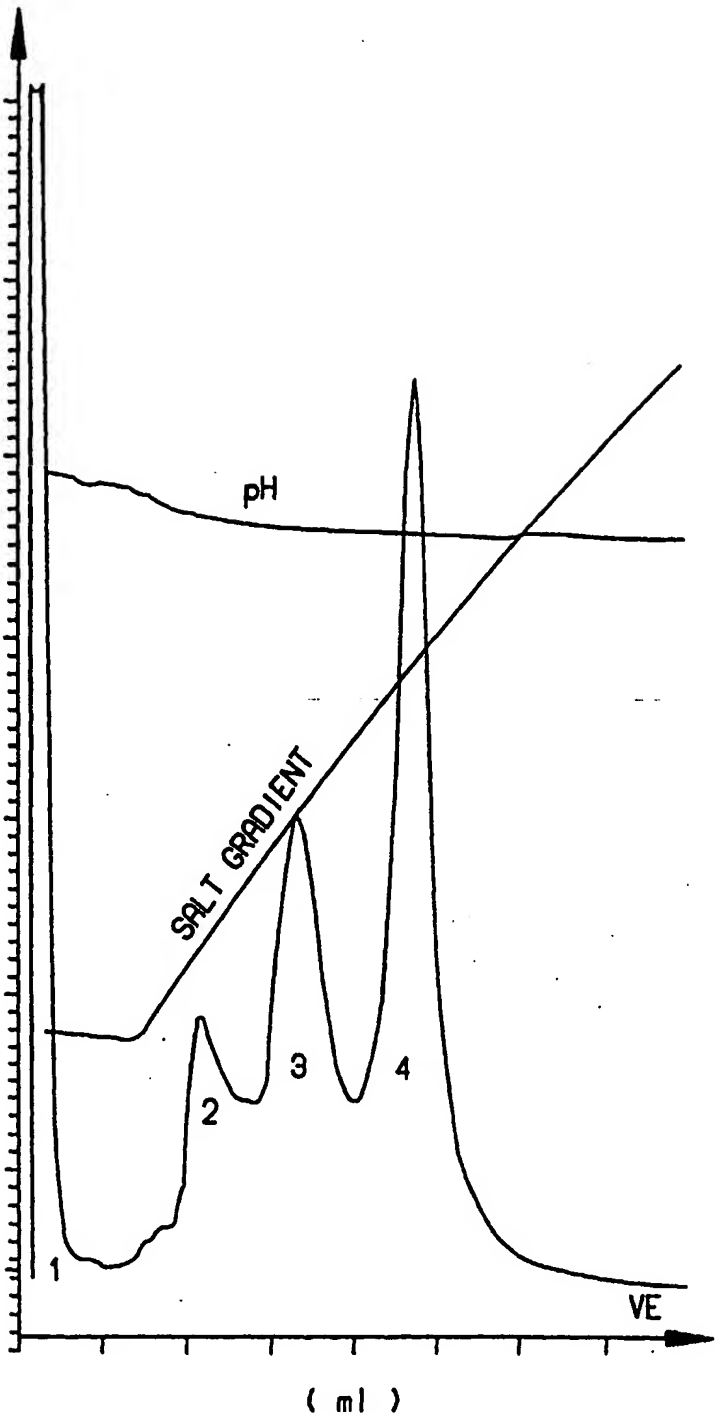

FIG. 1a

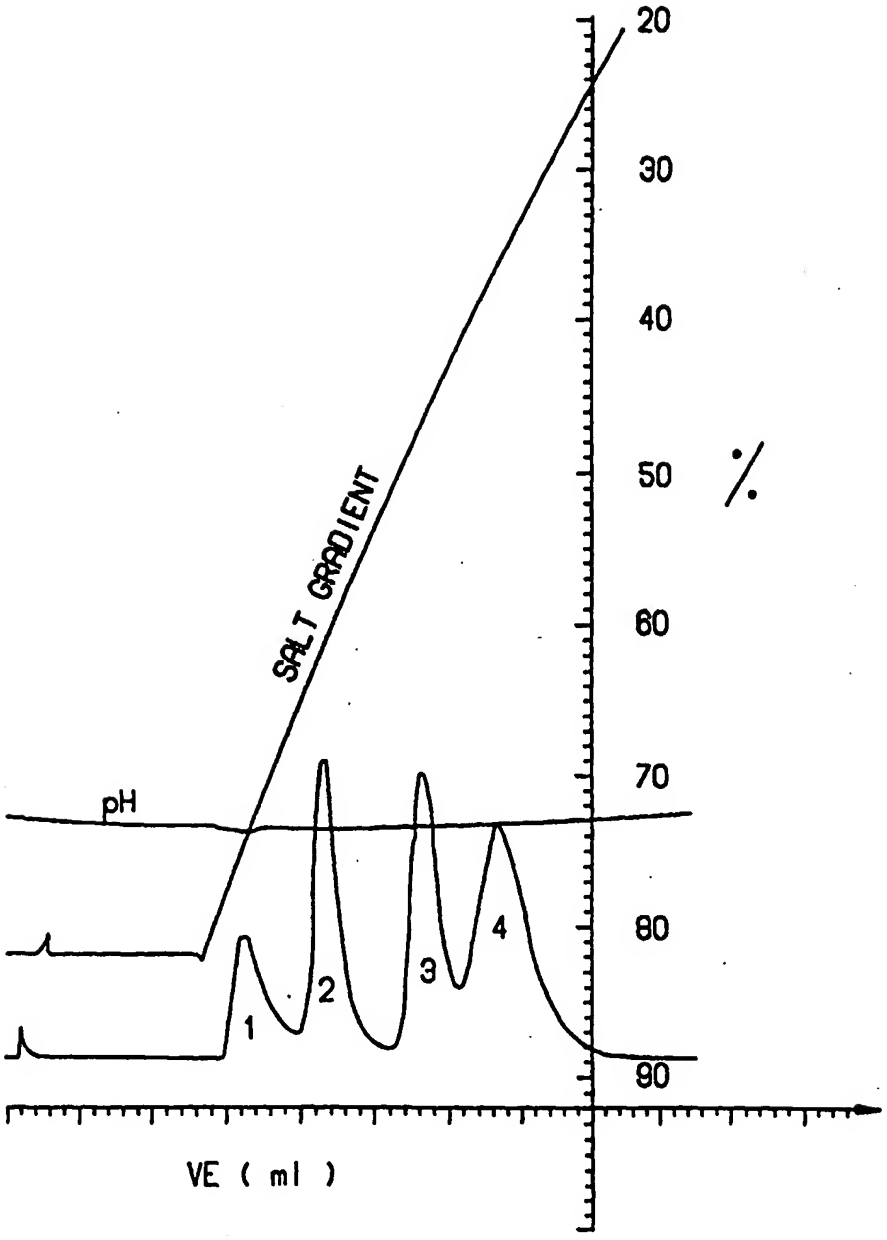

FIG. 1b

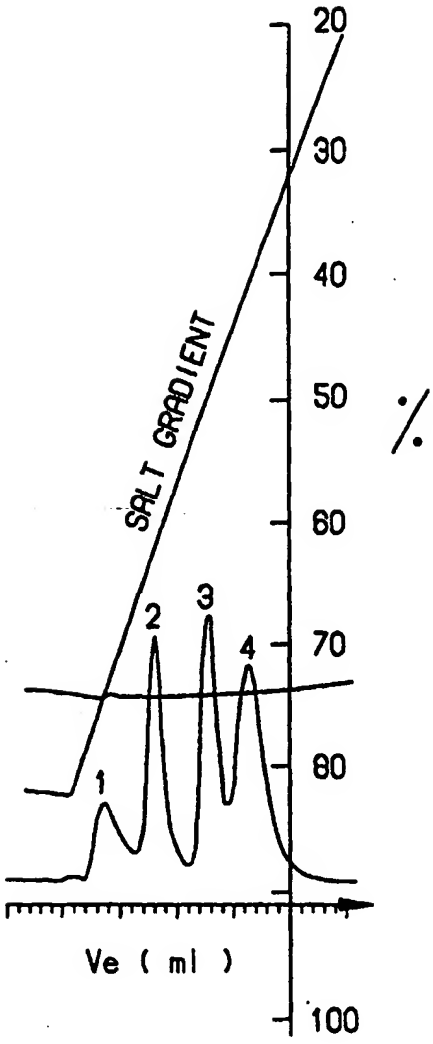
20
30
40
50
60
70
80
100
SALT GRADIENT
1
2
3
4
%
Ve (ml)

FIG. 2a

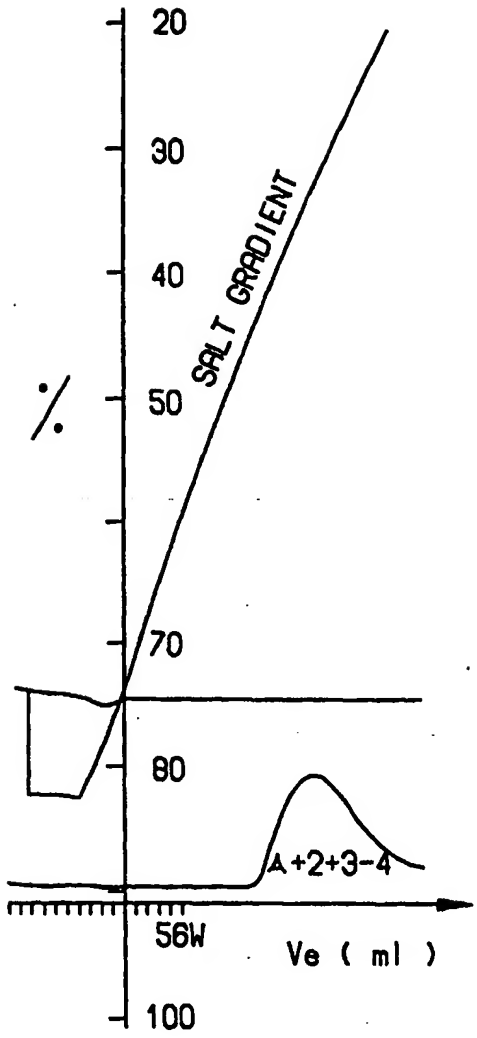
20
30
40
50
70
80
100
SALT GRADIENT
%
A+2+3-4
56W
Ve (ml)

FIG. 2b

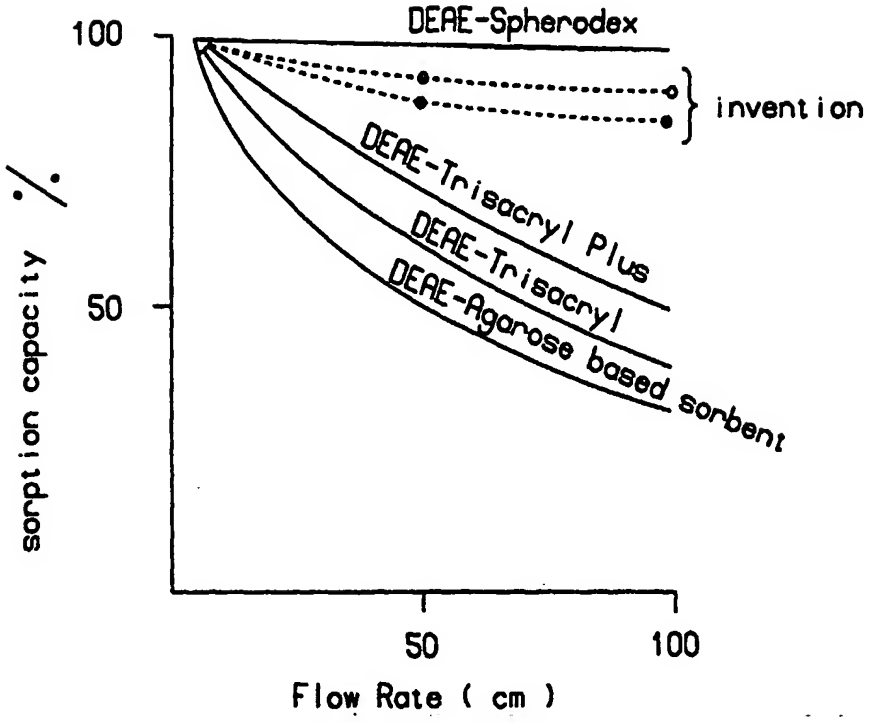
100
50
sorption capacity %
DEAE-Spherodex
invention
DEAE-Trisacryl Plus
DEAE-Trisacryl
DEAE-Agarose based sorbent
50
100
Flow Rate (cm)

FIG. 3a

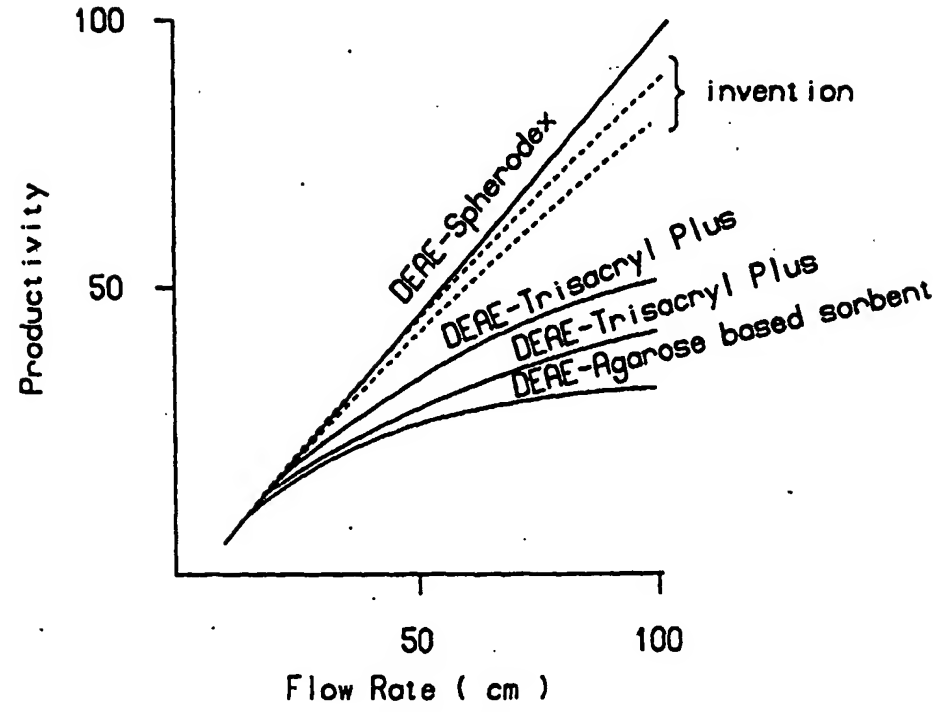
100
50
Productivity
invention
DEAE-Spherodex
DEAE-Trisacryl Plus
DEAE-Trisacryl Plus
DEAE-Agarose based sorbent
50
100
Flow Rate (cm)

FIG. 3b

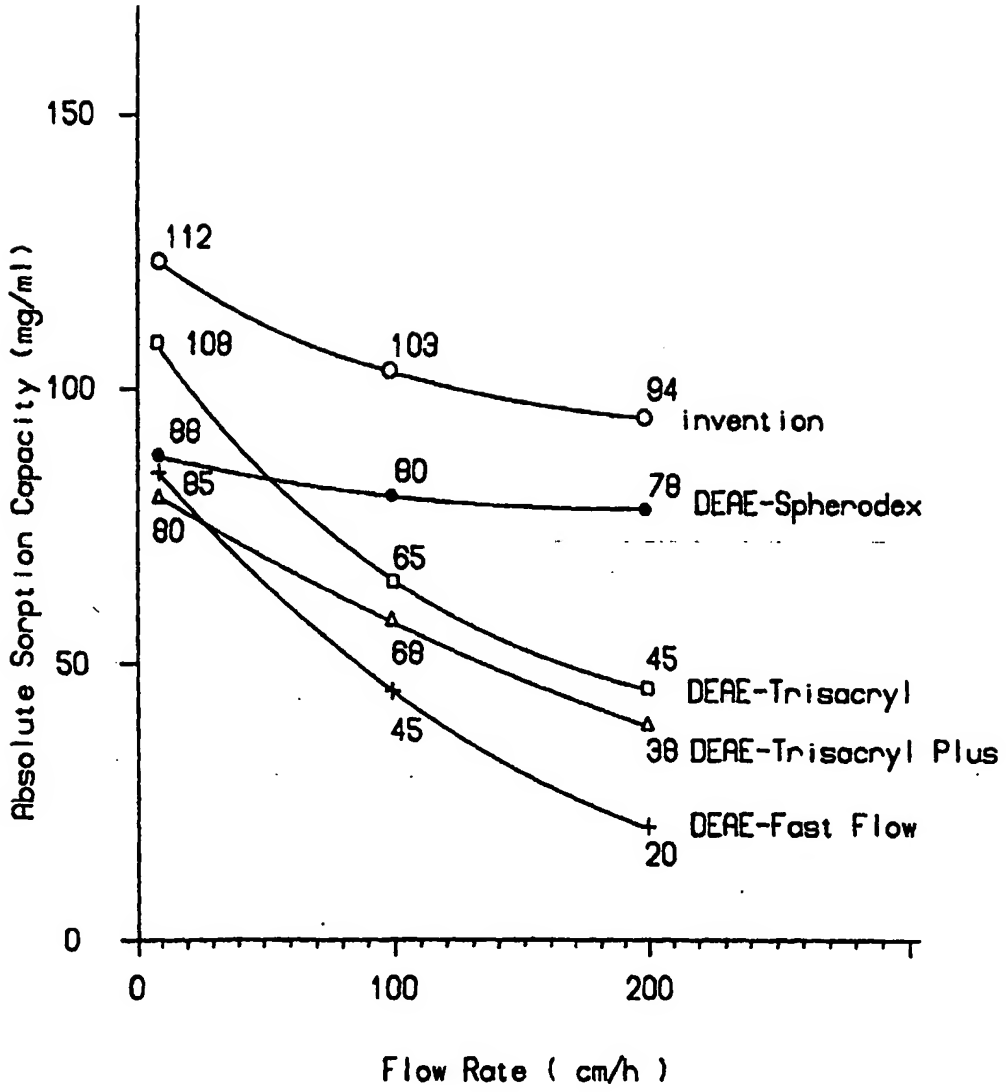
150
100
50
0
Absolute Sorption Capacity (mg/ml)
112
108
103
94
invention
88
85
80
80
78
DEAE-Spherodex
65
68
45
45
DEAE-Trisacryl
38
DEAE-Trisacryl Plus
DEAE-Fast Flow
20
0
100
200
Flow Rate (cm/h)

FIG. 4

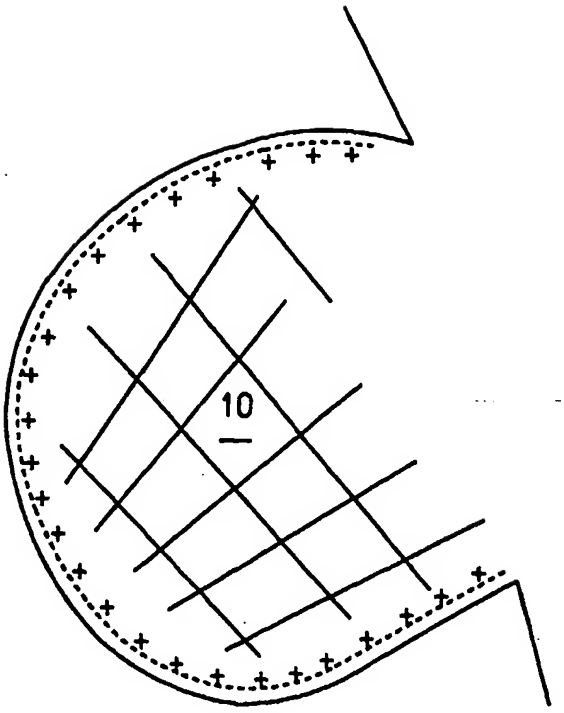

FIG. 5